ENVIRONMENTAL FORENSICS: AN INNOVATIVE TECHNIQUE USING BONE TO IDENTIFY MERCURY AND STABLE ISOTOPE LEVELS IN INTERNAL TISSUES OF WILDLIFE IN A CHANGING WESTERN ALASKA ENVIRONMENT

By

Bonita Hope Dainowski

RECOMMENDED:

Dr. Julie P. McIntyre

Dr. Paul W. Layer

Dr. Kriya L. Dunlap

Dr. Lawrence K. Duffy
Advisory Committee Chair

Dr. Thomas K. Green
Chair, Department of Chemistry and Biochemistry

APPROVED:

Dr. Paul W. Layer
Dean, College of Natural Science and Mathematics

Dr. Michael A. Castellini
Dean of Graduate School

May 2, 2017
Date

ENVIRONMENTAL FORENSICS: AN INNOVATIVE TECHNIQUE USING BONE TO IDENTIFY MERCURY AND STABLE ISOTOPE LEVELS IN INTERNAL TISSUES OF WILDLIFE IN A CHANGING WESTERN ALASKA ENVIRONMENT

A
DISSERTATION

Presented to the Faculty
of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy in Forensic Science: Interdisciplinary Program

By

Bonita Hope Dainowski, B.A., M.S.c

Fairbanks, AK

May 2017

Abstract

We evaluated if total mercury (THg) concentrations of keratin-based and bone-based tissues can predict THg concentrations in skeletal muscle, renal medulla, renal cortex, and liver. The THg concentration in matched tissues of 65 red foxes, *Vulpes vulpes*, from western Alaska was determined. Hair THg concentration had a significant positive correlation with liver, renal medulla, renal cortex, and muscle. The THg concentration is moderately predictive of THg concentration in the renal cortex and liver for these foxes based on R^2 values ($R^2 = 0.61$ and 0.63, respectively), but was not moderately predictive of THg for renal medulla $R^2 = 0.50$ and muscle $R^2 = 0.39$. Bone is weakly predictive of THg concentration in muscle ($R^2 = 0.40$), but not a reliable tissue to predict THg concentration in liver ($R^2 = 0.24$), renal cortex ($R^2 = 0.35$), or renal medulla ($R^2 = 0.25$).These results confirm the potential use of trapped animals, specifically foxes, as useful Arctic sentinel species to inform researchers about patterns in THg levels over time as industrialization of the Arctic continues.

Stable isotope analysis was also performed on the same red fox tissues from the first study. We examined stable carbon ($\delta^{13}C$) and nitrogen isotopes ($\delta^{15}N$) to 1) examine the lipid extraction process, 2) evaluate carbon and nitrogen correlations among tissues to establish stable isotope values for modern northern wild fox populations, 3) describe the C:N ratios in males and females, 4) establish trophic positions of free-ranging northern red foxes, and 5) to relate the wild red fox trophic level to potential mercury biomagnifications reported in a previous study. Hair, bone, muscle, liver, renal cortex and medulla tissues of the red fox were isotopically significantly different from each other. We found evidence that the Western Alaska red fox was eating a different diet based upon a lower trophic position than red foxes from other northern areas. We concluded that stable isotope data can help explain mercury concentration levels influenced by seasonal diet changes of the Alaska red fox.

In our third study, we took a forensic approach to look at the health and dietary indicators in museum preserved bone of red (*Vulpes vulpes*) and Arctic foxes (*Vulpes lagopus*) from the Yukon Territory in Canada. This study attempts to 1) measure the mercury (THg) concentration levels, 2) estimate a diet using carbon stable isotopes ($\delta^{13}C$) and 3) establish a trophic level using nitrogen stable isotopes ($\delta^{15}N$), from bones of these sentinel species. This study examines two Arctic foxes and three red foxes of unknown age and origin. Yukon Territory Arctic foxes THg concentrations were 0.017 and 0.025 mg/kg. The red foxes THg concentrations were 0.010, 0.036 and 0.073 mg/kg. The $\delta^{13}C$ levels were -21.13‰ and -21.36‰ for Arctic foxes and -20.05‰, -20.08‰, and -23.12‰ for red foxes. Their $\delta^{15}N$ levels were 5.59‰ and 7.22‰ for the Arctic foxes and 6.10‰, 6.57‰ and 6.66‰ for red foxes. These Arctic and red Yukon Territory foxes indicate a trophic level similar to Arctic terrestrial omnivores.

Dedication

In memory of my father
Benjamin Harold Dainowski
(1912-1986)
A true inspiration in my life

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to thank Mr. Jim Hemauer, my first undergraduate disability advisor, and Dr. Christy Turner, my first honors undergraduate advisor at Arizona State University, for their dedication, inspiration, encouragement and never ending belief in me to continue my studies and love of research in the sciences.

I would like to thank my Masters committee at Bournemouth University, England, for developing my interest in forensics.

I would like to thank all the trappers that submitted carcasses to ADF&G; and Dr. K. Beckmen for the assistance with sampling. I would like to thank Dr. J. Blake and assistants at the UAF Biological Research and Diagnostics Facility (BiRD) for the use of their necropsy suite for the collection of samples. I would also like to thank Megan Templeton for her help in coring the bones. Many thanks to Dr. S. Long of NIST for his efforts in working with myself to be able to certify bone meal (1486) for use as a THg SRM. Funding provided by the Alaska INBRE program. Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103395. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

I would like to thank my advisor, Dr. Larry Duffy, and committee member Dr. Julie McIntyre who have been with me through many challenging times during this PhD process. They never stopped supporting or believing in me. I would also like to thank my two other committee members, Dr. Paul Layer and Dr. Kriya Dunlap for their support and dedication in making this PhD possible.

I would like to thank Laura Bender and Shelly Baumann from the Graduate School for everything they have helped me with during all my years at UAF. They have been gracious and supportive.

General Introduction

A major issue affecting ecosystems globally is climate change. These changes impact animal populations and vegetation. Most omnivore animals consume more than one kind of prey, and each prey uptakes energy and nutrients as they move through their ecosystem. The red fox, *Vulpes vulpes,* is an opportunistic omnivore who inhabits most ecosystems across the Arctic and sub-Arctic; and thus can be affected by this changing environment. The Alaskan ecosystem with it's changing weather patterns and increase of industrialization and mining activities that release contaminants such as mercury into the environment, can upset an animal's food source (Gerlach et al., 2006). These type of changes could have an impact not only on the health of the ecosystem but also the health of the animals. Since the red fox consumes different prey from multiple and overlapping food chains, this research focuses on the association between diet and total mercury (THg) concentration in a Western Alaskan red fox population. The goal of this study is to identify toxicant and stable isotopes in multiple tissues of the fox in order to validate the use of hard and keratinized tissues for temporal and regional monitoring. Therefore, this "One Health" research study uses keratinized tissue (hair) and bone (femur) as markers to predict THg concentration levels and stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in soft tissues (kidney, liver, and muscle) in the red fox while accounting for age and sex.

Forensic toxicology and feeding ecology, as indicated by stable isotopes of $\delta^{13}C$ and $\delta^{15}N$, represents an interdisciplinary approach that integrates principles of biology, chemistry, forensics, anthropology, physiology and biomedicine. This research leads to information about feeding ecology and related toxicant exposure of a key sentinel species and can be applied to other keystone species. The association between feeding ecology and contaminant concentrations will be explored in the context of climate change.

Wildlife sentinels can be used as a proxy to monitor both ecosystem health and as a proxy to monitor health in Alaskan Natives and other subsistence users. Since patterns of Alaskan Native health and diet have shifted from a complete subsistence diet in pre-contact circumpolar populations to post-contact conditions that include "Western" foods for indigenous circumpolar populations (Coltrain et al., 2003; Fogel et al., 1997; Richards et al., 2003), along with social, cultural, and ecological changes, these health issues have become increasingly important in biomedical research (Meier-Augenstein and Fraser, 2008; Sephton et al., 2007). Therefore, to identify and understand dietary changes through time, it is necessary to understand the pathways in which fundamental changes in food systems occurs (Kellner and Schoeninger, 2007; McCutchan et al., 2003; Tykot, 2004). Changes in food ecosystems that influence an individual's diet, can also strengthen or weaken an individual's physical well-being and impact the health of individuals and communities (Loring and Duffy, 2011; Loring and Gerlach, 2009; Tykot, 2004; Wang et al., 2009).

The use of keratinized tissue, hair, is a non invasive approach for research and has been shown to be affected by season, infection, psychological stress, nutrition and genetics (Engel et al., 2010). Studies usually focus more on keratinized tissues from marine and aquatic biota, yet information on contaminant levels in terrestrial wildlife's keratinized tissues in circumpolar regions is less abundant (Gerlach et al., 2006). This keratinized tissue will be a good measurement not only for the health of the mammal through stable isotope studies but also as a temporal biomarker to evaluate potential effects and predict toxicants in soft tissues (Dietz et al., 2011; Holm et al., 2010). Predicting soft tissue concentrations could prove valuable in making stable isotope and toxicological assessments and move this effort beyond "monitoring" to data based adaptive management.

Few studies have included bone (Doyle, 1979) to predict toxicant levels in soft tissue from the Arctic, specifically Alaska (Keenleyside, 1998; Lobedell and Dekin, 1984). Bone plays a unique role in the pathway of pollutants and can be easily sampled and archived. Many toxicants are actively absorbed in bone mineral and then released later during the normal process of bone remodeling (McGowan, 1996). Toxicants can have both a direct effect on the bone itself and a latent effect on other organ systems when toxicants are released from bone long after initial exposure. Since bone collagen has a slow turnover period (Tieszen et al., 1983), we used it in our stable isotope research to infer red fox lifetime diet.

In the majority of the literature, the kidney is digested whole, both cortex and medulla together (Bilandzic et al., 2010; Cybulski et al., 2009; Dietz et al., 2000; Dip et al., 2001; Gutieb et al., 1998), before analysis of toxicants or freeze-dried and homogenized for stable isotope studies. Therefore, an innovation of this approach is separating the renal cortex from the medulla and analyzing each component of the kidney individually. Different structures in the kidney perform different functions and these different structures can accumulate toxicants at different rates and amounts (Aukland, 1989; Reidy and Rosenblum, 2009). Data from the fox indicates cortex has consistently higher THg concentration than medulla (~3:1) and this is an important consideration for monitoring and determining potential adverse effects (Dainowski et al., 2015).

The first approach is investigating forensic wildlife toxicology as it plays an important role in providing information about the biological effects of contaminants in the environment. Contaminants may have adverse effects on both wildlife and humans and, in many cases, may bioaccumulate and biomagnify. Understanding how contaminants move through food webs is imperative since a variety of terrestrial and marine wildlife serve as subsistence food for indigenous populations. We use a forensic approach for assessing THg concentrations over time (millennia) based on museum samples and use this information as a foundation for future assessments of THg in food webs (Murray et al., 2015). Therefore, Chapter 1 evaluated if total mercury (THg) concentrations of keratin-based and bone-based tissues can predict THg concentrations in skeletal muscle, renal medulla, renal cortex, and liver. The THg concentration in matched tissues of 65 red foxes, *Vulpes vulpes*, from western Alaska was determined. Hair THg concentration had a significant positive correlation with liver, renal medulla, renal cortex, and muscle. The THg concentration for males and females is moderately predictive of THg concentration in the renal cortex and liver for these foxes based on R^2 values ($R^2 = 0.61$ and 0.63, respectively). Bone is weakly predictive of THg concentration in muscle ($R^2 = 0.40$), but not a reliable tissue to predict THg concentration in liver ($R^2 = 0.24$), renal cortex ($R^2 = 0.35$), or renal medulla ($R^2 = 0.25$). These results confirm the potential use of trapped animals, specifically foxes, as useful Arctic sentinel species to inform researchers about patterns in THg levels over time as industrialization of the Arctic continues.

The second approach analyzes the same five tissues for stable isotopes ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to provide a feeding ecology assessment since THg concentrations often vary based on trophic interactions. Because THg can bioaccumulate and biomagnify, $\delta^{15}N$ is used to assess trophic level and $\delta^{13}C$ is used as markers of regional variability of marine vs. terrestrial prey for the red fox. Thus, Chapter 2 is the first study to analyze stable carbon and nitrogen isotopes in various tissues from one population of wild free-ranging red foxes (*Vulpes vulpes*) in Western Alaska for 1) to describe the C:N ratios in males and females, 2) to evaluate carbon and nitrogen correlations among various tissues with the objective of improving the baseline of stable isotope values for current wild fox populations for comparison with museum collections, 3) to relate the wild red fox trophic level to potential mercury biomagnifications reported in a previous THg study with the same red fox population. Hair, bone, muscle, liver, renal cortex and medulla tissues of the red fox were isotopically different.

The third approach uses forensic research to look at the paleo-health (mercury) and paleo-dietary (isotopes) indicators in preserved museum bone collagen of the red (*Vulpes vulpes*) and Arctic fox (*Vulpes lagopus)*, from Yukon Alaska. With climate changes affecting ecosystems in the Arctic, it is important to establish a baseline for toxicants and stable isotopes in order to predict any future changes that may affect not only wildlife health but that of the indigenous populations. In Chapter 3, we use the techniques from the previous two studies to 1) establish information on reconstructing a diet using carbon stable isotopes and 2) establish a trophic level using nitrogen stable isotopes, for these sentinel species. Because of the small sample sizes, two red fox bones, and three Arctic fox bones, as well as different bones (i.e. femur, tibia, mandible) being analyzed, and no indication of the sex of the foxes, it is not of any value to do any kind of statistical analysis. We did, however, present both THg and stable isotopes means and standard deviations to get a visual only perspective on what a diet might look like. The diet of the two Arctic foxes and two of the red foxes from the Yukon Territory Fossil Collection tend toward a salmon migration diet, and the third red fox toward a terrestrial mammal diet. The trophic levels indicate these red and Arctic foxes from the Yukon Territory are similar to omnivores, tending toward a slight salmon diet.

References

Aukland, K., 1989. Myogenic mechanisms in the kidney. Journal of Hypertension Supplement 7 (4), S71-76.

Bilandzic, N., Dezdek, D., Sedak, M., Dokic, M., Solomun, B., Varenina, I., Knezevic, Z., Slavica, A., 2010. Concentrations of Trace Elements in Tissues of Red Fox (*Vulpes vulpes*) and Stone Marten (*Martes foina*) from Suburban and Rural Areas in Croatia Bull. Environ. Contam. Toxicol. 85, 486-491.

Coltrain, J.B., Hayes, M.G., O'Rourke, D.H., 2003. Sealing, whaling and caribou: the skeletal isotope chemistry of Eastern Arctic foragers. J. Archaeol. Sci. 00, 1-19.

Cybulski, W., Chalabis-Mazurek, A., Jakubczak, A., Jarosz, L., Kostro, K., Kursa, K., 2009. Content of lead, cadmium, and mercury in the liver and kidneys of silver foxes (*Vulpes vulpes*) in relation to age and reproduction disorders. Bull. Vet. Inst. Pulawy 53, 65-69.

Dainowski, B.H., Duffy, L.K., McIntyre, J., Jones, P., 2015. Hair and bone as predictors of tissular mercury concentration in the Western Alaska Red Fox, *Vulpes vulpes*. Sci. Total Environ. (518-519), 526-533.

Dietz, R., Riget, F., Born, E.W., 2000. Geographical differences of zinc, cadmium, mercury and selenium in polar bears (*Ursus maritimus*) from Greenland. Sci. Total Environ. 245, 25-47.

Dietz, R., Born, E.W., Riget, F., Aubail, A., Sonne, C., Drimmie, R., Basu, N., 2011. Temporal Trends and Future Predictions of Mercury Concentrations in Northwest Greenland Polar Bear (*Ursus maritimus*) Hair. Environ. Sci. Technol. 45, 1458-1465.

Dip, R., Stieger, C., Deplazes, P., Hegglin, D., Muller, U., Dafflon, O., Koch, H., Naegeli, H., 2001. Comparison of Heavy Metal Concentrations in Tissues of Red Foxes from Adjacent Urban, Suburban, and Rural Areas. Archi. Environ. Contam. Toxicol. 40, 551-556.

Doyle, J.J., 1979. Toxic and Essential Elements in Bone - A Review. J. Anim. Sci. 49, 482-497.

Engel, G., O'Hara, T.M., Cardona-Marek, T., Heidrich, J., Chalise, M.K., Kyes, R., Jones-Engel, L., 2010. Synanthropic Primates in Asia: Potential Sentinels for Environmental Toxins. Am. J. Phys. Anthropol. 140, 453-460.

Fogel, M.L., Tuross, N., Johnson, B.J., Miller, G.H., 1997. Biogeochemical record of ancient humans. Org. Geochem. 27 (5/6), 275-287.

Gerlach, S.C., Duffy, L.K., Murray, M.S., Bowers, P.M., Adams, R., Verbrugge, D.A., 2006. An exploratory study of total mercury levels in archaeological caribou hair from northwest Alaska. Chemosphere 65, 1909-1914.

Gutieb, A.C., Kranz, A., Nechay, G., Toman, A., 1998. Heavy Metal Concentrations in Livers and Kidneys of the Otter (*Lutra lutra*) from Central Europe. Bull. Environ. Contam. Toxicol. 60, 273-279.

Holm, E., Gwynn, J., Zaborska, A., Gafvert, T., Roos, P., Henricsson, F., 2010. Hair and feathers as indicator of internal contamination of 210Po and 210Pb. In: Holm E, editor. Roskilde: Nordic nuclear safety research. p 1-27.

Keenleyside, A., 1998. Skeletal Evidence of Health and Disease in Pre-Contact Alaskan Eskimos and Aleuts. Am. J. Phys. Anthropol. 107, 51-70.

Kellner, C.M., Schoeninger, M.J., 2007. A Simple Carbon Isotope Model for Reconstructing Prehistoric Human Diet. Am. J. Phys. Anthropol. 133, 1112-1127.

Lobedell, J.E., Dekin, A.A., 1984. The Frozen Family from the Utqiagvik Site, Barrow, Alaska - Papers from a Symposium - Introduction. Arctic Anthropol. 21, 1-4.

Loring, P.A., Duffy, L.K., 2011. Managing environmental risks: the benefits of a place-based approach. Rural Remote Health 11, 1800.

Loring, P.A., Gerlach, S.C., 2009. Food, culture, and human health in Alaska: an integrative health approach to food security. Environ. Sci. Policy 12, 466-478.

McCutchan, J.H., Lewis, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. OIKOS 102, 378-390.

McGowan, J.A., 1996. Bone: Target and source of environmental pollutant exposure. Otolaryngology - Head Neck Surg. 114 (2), 220-223.

Meier-Augenstein, W., Fraser, I.. 2008. Forensic Isotope analysis leads to identification of a mutilated murder victim. Sci. Justice 48, 153-159.

Murray, M.S., McRoy, C.P., Duffy, L.K., Hirons, A.C., Schaaf, J.M., Trocine, R.P., Trefry, J., 2015. Biogeochemical analysis of ancient Pacific cod bone suggest Hg bioaccumulation was linked to paleo sea level rise and climate change. Front. Environ. Sci. 3 (8), 1-8.

Reidy, K.J., Rosenblum, N.D., 2009. Cell and Molecular Biology of Kidney Development. Seminars in Nephrology 29 (4), 321-337.

Richards, M.P., Schulting, R.J., Hedges, R.E.M., 2003. Sharp shift in diet at onset of Neolithic. Nature. p 366.

Sephton, M.A., Meredith, W., Sun, C.G., Snape, C.E., 2007. Biomedical and Forensic Applications of Combined Catalytic Hydrogenation-Stable Isotope Ratio Analysis. Anal. Chem. Insights 2, 37-42.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., Slade, N.A., 1983. Fractionation and turnover for δ13C analysis of diet in animal tissues: Implications for δ13C analysis of diet. Ecology 57, 32-37.

Tykot, R.H., 2004. Stable isotopes and diet: You are what you eat. Proceeding of the International School of Physics "Enrico Fermi" IOS Press: Amsterdam.

Wang, N., Wu ,Y., Zhang, J., 2009. Comparison and unification of carbon stable isotope ratios in specific aquatic biota. Commun. Nonlinear Sci. Numer. Simul. 14, 2502-2506.

Chapter 1: Hair and bone as predictors of tissular mercury concentration in the Western Alaska red fox, *Vulpes vulpes*[1]

1.1 Abstract

We evaluated if total mercury (THg) concentrations of keratin-based and bone-based tissues can predict THg concentrations in skeletal muscle, renal medulla, renal cortex, and liver. The THg concentration in matched tissues of 65 red foxes, *Vulpes vulpes*, from western Alaska was determined. Hair THg concentration had a significant positive correlation with liver, renal medulla, renal cortex, and muscle. The THg concentration for males and females is moderately predictive of THg concentration in the renal cortex and liver for these foxes based on R^2 values (R^2 = 0.61 and 0.63, respectively). Bone is weakly predictive of THg concentration in muscle (R^2 = 0.40), but not a reliable tissue to predict THg concentration in liver (R^2 = 0.24), renal cortex (R^2 = 0.35), or renal medulla (R^2 = 0.25). These results confirm the potential use of trapped animals, specifically foxes, as useful Arctic sentinel species to inform researchers about patterns in THg levels over time as industrialization of the Arctic continues.

[1]Dainowski BH, Duffy LK, McIntyre J, Jones P. 2015. Hair and bone as predictors of tissular mercury concentration in the Western Alaska red fox, *Vulpes vulpes*. Sci. Total Environ. 15 (518-519), 526-533.

1.2 Introduction

Globally, mercury (Hg) is a naturally occurring element found throughout the environment in air, water, and soil. It exists in several forms: elemental (metallic mercury), inorganic (mercuric chloride) and organic (methylmercury) compounds (Clarkson and Magos, 2006). It is released into the atmosphere and water by volcanic activity, weathering of rocks; and, anthropogenic mercury is through industry emissions from agriculture, manufacturing, and mining (Bilandzic et al., 2010; Clarkson, 1997; Clarkson and Magos, 2006; Kalisinska et al., 2012). In ecotoxicological studies, total mercury (THg) is mainly determined as the sum of organic (mostly as methylmercury) and inorganic mercury (Eisler, 1987). Many organisms are exposed through the food web and are sensitive to the effects of mercury, especially monomethylmercury ($MeHg^+$) (Brookens et al., 2008; Dietz et al., 2009). The degree of food web biomagnification of $MeHg^+$ depends on dietary patterns, as well as age and sex (Bilandzic et al., 2010; Clarkson and Magos, 2006; Cumbie and Jenkins, 1974; Knott et al., 2011a; Wren, 1986).

With increased release of Hg into the environment and potential methylation, tissue concentrations in foxes will likely increase; therefore, it is important to continue biomonitoring Hg concentrations in terrestrial ecosystems over time in medium-sized carnivore mammals especially ones that are numerous and widely distributed ones like the red fox (Dunlap et al., 2007). Mercury methylation occurs in aquatic environments and $MeHg^+$ is absorbed mainly from the gastrointestinal tract of some organisms and then distributes to and accumulates in some organs, including muscle, kidney, liver, and the brain (Bilandzic et al., 2010; Castoldi et al., 2001; Clarkson, 1997; Cybulski et al., 2009; Davis et al., 1994; Dehn et al., 2006; Lopez-Artiguez et al., 1995; Millan et al., 2008; Wren, 1986). In contrast to $MeHg^+$, elemental Hg is poorly absorbed by the gastrointestinal tract (Wolfe et al., 1998; Wiener et al., 2003) but may be methylated in the rumen of herbivores, absorbed and redistributed (Duffy et al., 2005; Lokken et al., 2009).

The toxicity of $MeHg^+$ and its accumulation in aquatic biota, humans, and domestic animals is well documented (Adbdulla and Chmielnicka, 1990; Becker, 2000; Brookens et al., 2008; Cybulski et al., 2009; Dunlap et al., 2007; Gaden et al., 2009; Knott et al., 2011b; Lopez-Artiguez et al., 1995). On the other hand, relatively little is understood about these processes in wild terrestrial mammals of the Arctic (Dehn et al., 2006; Gamberg et al., 2005). Studies have shown a correlation of THg concentrations among various tissues (Cumbie, 1975; Dip et al., 2001; Kalisinska et al., 2009; Piskorová et al., 2003; Wren, 1986). Muscle, liver and kidney are often used as bioindicators to assess biological variation among cohorts, individuals, and populations (Alonso et al., 2004; Becker and Wise, 2006; Brookens et al., 2008; Gutleb et al., 1998; McGrew et al., 2013; Yamamoto et al., 1987). Hair is a well documented valuable matrix for some heavy metal contaminant monitoring and is a relatively metabolically inactive tissue that represents a route of elimination (Beckmen et al., 2002; Duffy et al., 2005; Yin et al., 2007). For example, hair is a good indicator of THg in blood as it incorporates $MeHg^+$ from the blood during growth and has been shown to correlate with levels in the brain (Cernichiari et al., 1995; Dietz et al., 2009, 2011).

Moreover, the slower decay rates of hair, as well as bone, in the soil make them more readily available than soft tissues for analysis. For example, museum collections often contain samples of bone and pelts. However, few researchers have used bone as a predictor for soft tissue THg concentrations. Interestingly, two reports were found on Hg that was studied in wild carnivore bones, including in the red fox (Lanocha et al., 2012; Millan et al., 2008). Bone is an important tissue because it accumulates some elemental toxicants (Doyle, 1979; Halffman, 2009; Lehner, 2012; Nielsen-Marsh and Hedges, 1999). Studies of bone are of great importance for the prevention of diseases caused by trace element imbalance (Klepinger, 1984; Kwapulinski et al., 1995; Martinez-Garcia et al., 2005; Takata and Saiki, 2004).

The red fox, *Vulpes vulpes*, is a widely distributed, medium-sized canid, which adapts to a variety of environmental conditions (Aubry et al., 2009; Corsolini et al., 1999; Kiener and Zaitsev, 2010). Red foxes use a wide trophic niche driven by local and seasonal food availability, including, garbage, small rodents, invertebrates, and small amounts of fruit (Cavallini and Lovari, 1991; Ciampalini and Lovari, 1985; Corsolini et al., 1999; Doncaster and MacDonald, 1991; Goldyn et al., 2003; Hersteinsson and Macdonald, 1992; Hewson and Kolb, 1975; Jones and Theberge, 1982; Kalisinska et al., 2009; Lovari et al., 1994; McIntosh, 1963; Saunders et al., 1993; von Schantz, 1980). The red fox is an ecological generalist, who sometimes consumes some nutritionally inferior food items (Corsolini et al., 1999; Dell'Arte et al., 2007; Kamler and Ballard, 2002).

We evaluated mercury exposure in the wild population of red foxes, *V. vulpes*, of Bethel Alaska. The focus of this paper is to determine if bone and hair can be reliable predictors for THg concentrations in the liver, muscle, renal cortex and renal medulla. In addition this study adds new information on the kidney because the kidney is usually not separated in research. Finally we compare our findings with those of other contaminant research studies using foxes from other northern latitudes.

1.3 Materials and Methods

1.3.1 *Study area*

The Kuskokwim River (Figure 1.1), fed by mountain streams which feed into the main river, has been known to have high concentrations of naturally occurring mercury (Rytuba, 2003). Also there are several historical mines in the Bethel region. The Red Devil Mine is one in particular that has abandoned tailings which are eroding into the river (Bailey and Gray, 1997).

1.3.2 *Carcass collection and processing*

A total of 200 red fox, *V. vulpes*, carcasses were donated during the period of November 2010 through February 2011. Trappers anonymously and voluntarily provided carcasses to the Alaska Department of Fish and Game (ADF&G) in Bethel, Alaska. Carcasses remained frozen and were stored outside at the ADF&G office (temperature was continuously below 0°C).

Frozen carcasses were transported to the University of Alaska Fairbanks (UAF) where they were stored at -20°C. Carcasses were partially thawed and sub-samples of liver, one whole kidney, femur, muscle, and rear paw with fur were collected into Whirl-Pak™ bags and stored at -20°C or -80°C (femurs). All samples were collected from the right side of the carcass using stainless steel scalpels or scissors. Only foxes with all five available tissues were analyzed for mercury. Based on these criteria, 65 foxes were used in this study, 35 males and 30 females.

Tissues were prepared for lyophilization according to the following procedures, using disposable stainless steel scalpels (new scalpel for each sample).

1.3.3 *Liver, muscle, renal cortex and renal medulla*

Approximately 14 g of frozen liver was cut into small pieces (~1 cm^3). The entire quadricep muscle was removed from the femur bone while partially frozen, and cut into small pieces. For dissection of cortex and medulla, kidneys were kept partially frozen on a clean stainless steel tray placed on ice. All instruments used to separate renal cortex and medulla (scalpels and trays) were also kept at -20°C before use. All tissues were placed into individual pre-weighed Whirl-Pak™ bags and lyophilized for 72 hours (Labconco FreeZone 4.5™ Freeze Dry System). Tissue mass was determined before and after lyophilization.

1.3.4 *Hair and bone*

Hair was collected from the right rear paw using a Wahl stainless steel trimmer (carbon blades) and stainless steel scissors. Blades were thoroughly cleaned between each sample. Hair was cleaned and dried following the methods of Castellini et al. (2012).

Bones were cored under a fume hood while wearing a 3 M™ particulate respirator N95. The bones were cored using a Dremel™ glass diamond drilling bit, 1/4″ (6.350 mm), 663DR, drill speed #23. Each femur was drilled completely through the shaft in three locations to produce six cores (Figure 1.2). The periosteum and any trabecular bone were removed using a Dremel™ glass diamond taper point sander 3/32″ (2.381 mm), #7144. Total core mass was determined for each individual for a total wet weight (ww). The cores were stored at -20°C in acid-washed (5% HNO_3) vials prior to degreasing.

Prior to mercury analysis, bone cores were degreased with a series of chloroform treatments in a chemical fume hood (Aerssens et al., 1998; Bell et al., 2001; Dwek, 2010; Schutkowski and Herrmann, 1999; Zwanziger, 1989). Enough chloroform (Chloroform, Reagent ACS, Sciencelab.com) was added to each vial to completely cover bone core samples. Each vial was then loosely capped. Samples were treated for a total of 16 hours, changing chloroform after every 8 hour chloroform treatment, samples were rinsed several times with ultrapure water. Zwanziger (1989) suggested that freeze drying bone results in decreased THg concentrations, thus degreased samples were air-dried in the fume hood for four days. Bone was homogenized for analysis using a Wig-L-Bug™ (Crescent Dental Company Chicago) with a 9.5 mm stainless steel ball bearing (~10s).

1.3.5 *Total mercury (THg) concentration determination*

Approximately 16-20 mg of homogenized dry tissue (hair, renal cortex, renal medulla, muscle, liver) was analyzed for THg using a 2-cell DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, Connecticut, USA) according to USA EPA method 7473, with a minimum detection limit of 0.037 μg/g (Knott et al., 2011a; Lieske et al., 2011). Approximately 30 mg of homogenized powdered bone was analyzed for THg using a 3-cell low detection DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, Connecticut, USA; USA EPA method 7473). The low detection DMA-80 was calibrated using a 6 point linear calibration curve from 0.25 ng to 6.00 ng; $R^2 = 0.9999$, resulting in a minimum detection limit of 0.008 μg/g. All analytical runs included measurement of blanks, liquid standards (1 μg/g, 0.1 μg/g, 0.01 μg/g) and certified reference materials (IAEA-085, IAEA-086, DORM3, DOLT4) depending on tissue (Table 1.1).

1.3.6 *Statistical analyses*

Statistical analyses were performed using the R statistical software package (R Development Core Team, 2011). T-tests were used to compare mean THg concentrations between males and females for all tissues, as well as mean concentration between kidney cortex and kidney medulla. Pearson's correlation statistic was used to estimate the correlation between the two kidney tissues. Linear regression was used to investigate the relationship between hair and bone THg concentrations. Of particular interest was to determine whether hair and/or bone THg concentrations can be used to predict soft tissue, specifically muscle, liver, renal cortex, and renal medulla THg concentrations. Sex was also included as a predictor in all regression models to investigate possible differences in these relationships between males and females.

Models considering hair and bone THg predictors on each soft tissue response were fit separately. Each model first included a term for interaction between sex and THg concentration of hair/bone. If the interaction term was not significant, models were refit without it and the main effects of sex and hair/bone THg were tested. Nonsignificant main effects were then removed, resulting in a final model. Each fitted model was used to compute pointwise 95% prediction intervals for predicting soft tissue THg from hair/bone THg. Significance of all effects was determined using alpha = 0.05.

Residuals from the fitted model were examined to validate model assumptions. A log transformation on the response was employed if residuals showed evidence of nonnormality or nonconstant variance. Standardized residuals having an absolute value greater than three were identified as outliers and removed from the analysis.

R^2 values were used to evaluate the strength and usefulness of models for predicting soft tissue THg concentrations. Model evaluations were based on categories reported by O'Hara et al. (2008), where $R^2 \leq 0.35$ indicates no meaningful predictive ability, R^2 between 0.36 and 0.55 indicates weakly predictive ability, R^2 between 0.56 and 0.75 indicates moderately predictive ability, and $R^2 \geq 0.75$ indicates strongly predictive ability.

1.4 Results

Means ± standard deviations, minimums, maximums, and medians for each tissue type, as well as summary statistics for each tissue type separated by sex are reported in Table 1.2. p-values of t-tests comparing mean concentrations between males and females showed no significant differences. A significant difference was found between mean THg concentrations in kidney cortex and kidney medulla (p-value approximately zero). Pearson's correlation between concentration of kidney cortex and kidney medulla was 0.813.

Regression models found no significant interactions between sex and hair/bone THg concentrations for any of the soft tissues. The hair/bone main effects were significant in each regression model, indicating that hair/bone THg concentrations can be used to predict soft tissue THg concentrations. The main effect for sex was not significant in any of the regression models, suggesting that the relationship between soft tissue THg concentrations and hair/bone THg concentrations is the same for male and female foxes. Results of regression analyses are shown in Table 1.3. Fitted regression models with 95% prediction intervals are displayed in Figures 1.3 and 1.4.

Hair THg concentration is a moderate predictor of renal cortex ($R^2 = 0.61$) and liver ($R^2 = 0.63$) THg concentration. Hair THg concentration is weakly predictive of renal medulla ($R^2 = 0.50$) and muscle ($R^2 = 0.39$) THg concentrations. Bone THg concentration has no meaningful predictive value for liver ($R^2 = 0.24$), renal cortex ($R^2 = 0.35$), or renal medulla ($R^2 = 0.25$) THg concentrations. Bone THg concentration is weakly predictive of muscle ($R^2 = 0.40$) THg concentration.

In the regression analysis of both hair and bone, two outliers were identified and removed. However, the removal of these outliers did not change the results of regression models. Responses for models using bone THg concentration as a predictor were log transformed due to evidence of nonconstant variance.

1.5 Discussion

Many studies have focused on characterizing mercury levels in fox populations and establishing differences between sex and age groups, e.g. Kiener and Zaitsev (2010) and Lanocha et al. (2014). Mercury concentrations in terrestrial mammals occasionally show a wide intraspecific variability (Duffy et al., 2005; Kalisinska et al., 2009) influenced by natural environmental differences. Mercury concentrations in livers from red foxes, *V. vulpes*, from the Province of Siena in Italy were shown to differ in relation to sex and age (Corsolini et al., 1999). The difference in hepatic THg concentrations between foxes from Italy and Alaska based on a geochemical background is ~0.16 vs ~1.20 mg/kg dw. Their study differed from ours in regard to sex and age of the fox. We did not consider age in our study as we had very few foxes older than two. In addition, our study found no significant difference in male and female liver mean THg concentrations (males are 1.261 mg/kg dw; females are 1.185 mg/kg dw) as compared to Corsolini et al. (1999) study (yearling males avg. 0.18 mg/kg dw and adult males avg. 0.13 mg/kg dw; yearling females avg. 0.14 mg/kg dw and adult females avg. 0.16 mg/kg dw). The higher THg concentrations in liver of Alaska foxes may indicate they are eating at a higher trophic level, as Dehn et al. (2006) showed a similar result with polar bears, (*Ursus maritimus*); stable isotope studies could support this.

In Poland, Cybulski et al. (2009) assessed mercury levels in livers and kidneys of *V. vulpes*. The outer portion of the kidney which consists of the nephrons is the cortex and the inner portion which consists of the pyramids is the medulla (Boundless, 2014). Nephrons, the functional structure in the kidney, filter the blood to regulate chemical concentrations to produce the urine (Boundless, 2014). After this initial filtering, the waste passes deep into medullary pyramids producing urine to be passed to the bladder (Boundless, 2014). Their study differed from ours in that they studied foxes from a fur fox farm where our foxes were from their natural habitat. Cybulski and colleagues reported liver Hg concentrations as 0.257 ± 0.3403 mg/kg dw and kidneys as 0.600 ± 1.1112 mg/kg dw. Their research also used whole kidney where our research sub-sampled the renal cortex and renal medulla. We did not use whole kidney in our analysis as it may contain other contaminants such as blood and the waste products from the blood, which may interfere with THg analysis. Our study reported the average THg concentrations for all red fox livers analyzed as 1.226 mg/kg dw, renal cortex having 2.122 mg/kg dw, and renal medulla having 1.177 mg/kg dw, which are higher than that of the fox fur farm.

Samples of muscles, kidneys, and livers of red foxes, *V. vulpes*, were collected in Central region of Slovak Republic, an emissions contaminated area (Piskorová et al., 2003). This study indicated mercury levels between 0.06 and 1.43 mg/kg, in the tissues from wild foxes. In our study, our values are similar to their upper range. The muscle we used was the quadricep in contrast to their *m. semimebranosus* muscle. In addition, their numbers of samples were much smaller than in our study. We used 65 samples in contrast to their samples of 12 kidneys, eight livers and one muscle. Another difference is the use of whole kidney in the Piskorová et al. (2003) study, while we divided the kidney into renal cortex and renal medulla. Their study showed THg concentration level averages in liver as 0.22 mg/kg, kidney as 0.63 mg/kg, and muscle as 0.013 mg/kg. The Piskorová et al. (2003) study indicated their results in wet weight (ww). Changing our averages to ww by assuming a 75% hydration, our THg concentrations were higher as follows: 2.780, 0.308, renal cortex 0.484, and renal medulla 0.170 mg/kg ww, respectively.

In another study of the red fox, *V. vulpes*, the renal cortex, liver, and muscle was analyzed for mercury concentrations to determine if these mercury levels can be used as a bioindicator of toxic metal accumulation in urbanized habitats in Croatia (Bilandzic et al., 2010). Their results found that THg concentration values were slightly higher in rural areas of Croatia. This was the only study found, to date, that used the renal cortex and not the entire kidney. Their samples sizes were again small, 12 red fox for the suburban study and 16 red fox for the rural study. Their THg concentration averages for suburban and rural red fox is as follows: muscle (name not reported) 0.007 mg/kg ww and 0.004 mg/kg ww, liver 0.025 mg/kg ww and 0.009 mg/kg ww, and renal cortex 0.064 mg/kg ww and 0.032 μg/g ww, respectively. Table 2 reports our dry weight data and suggests the rural Alaska red fox have higher THg concentrations in their tissue than the Croatia foxes in Bilandzic et al.'s (2010) study.

Two European studies reported on THg concentrations that were found in wild red fox bones (Lanocha et al., 2012; Millan et al., 2008). In the compact bone tissue reported, the results showed 0.0054 mg/kg dw (ranges 0.0012–0.0226) and 0.012 mg/kg dw (ranges nd-0.038) respectively (Lanocha et al., 2012; Millan et al., 2008). This is in the same range as our Alaskan red foxes reporting mean THg for compact bone as 0.004 mg/kg with a range of 0.001 to 0.010 mg/kg (Table 1.2).

In general, with the exception of farmed foxes, the wild red foxes of Alaska, living in a relatively pristine watershed ecosystem, have higher natural THg concentration levels in their tissues than those reported in highly populated Europe where the environment is patchy. The more rural and intact natural environment of rural Alaska may allow the red fox to feed higher on the food chain, with an abundance of fish and small mammals.

1.6 Conclusion

This is the first data set reported for the red fox in Alaska in which THg concentrations for several tissues from the same animal were compared. Our findings support the hypothesis, in part, that THg concentration in hair is correlated across tissues and these correlations may be strong enough so one can predict THg concentrations in some tissues, specifically skeletal muscle, renal medulla, renal cortex, and liver. THg concentration in hair had a significant linear relationship to THg concentration in liver, renal cortex, renal medulla, and muscle for both males and females. Since hair is a good matrix to predict THg concentration in the renal cortex and liver for red foxes, hair samples from foxes could be used to indicate approximate liver and renal THg concentrations.

1.7 Acknowledgments

We would like to thank all the trappers that submitted carcasses to ADF&G; and Dr. Kimberlee Beckmen for the assistance with sampling. We would like to thank Dr. John Blake and assistants at the UAF Biolog- ical Research and Diagnostics Facility (BiRD) for the use of their necrop- sy suite for the collection of samples. I would also like to thank Megan Templeton for her help in coring the bones. Many thanks to Dr. Stephen Long of NIST for his efforts in working with the author to be able to cer- tify bone meal (1486) for use as a THg SRM. Funding provided by the Alaska INBRE program. Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103395. The content is solely the responsibility of the authors and does not nec- essarily represent the official views of the National Institutes of Health.

1.8 References

Adbdulla, M., Chmielnicka, J., 1990. New aspects on the distribution and metabolism of essential trace elements after dietary exposure to toxic metals. Biol. Trace Elem. Res. 23, 25-51.

Aerssens, J., Boonen, S., Lowet, G., Dequeker, J., 1998. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. Endocrinology 139, 663-670.

Alonso, M.L., Montana, F.P., Miranda, M., Castillo, C., Hernandez, J., Benedito, J.L., 2004. Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. BioMetals 17, 389-397.

Aubry, K.B., Statham, M.J., Sacks, B.N., Perrines, J.D., Wisely, S.M., 2009. Phylogeography of the North American red fox: vicariance in Pleistocene forest refugia. Mol. Ecol. 18, 2668-2686.

Bailey, E.A., Gray, J.E., 1997. Mercury in the terrestrial environment. Kuskokwim Mountains region, southwestern Alaska. In: Dumoulin, J.A., Gray, J.E. (Eds.), Geologic Studies in Alaska by the U.S. Geological Survey, 1995. U.S. Geological Survey Professional Paper 1574, pp. 41-56.

Becker, P.R., 2000. Concentrations of chlorinated hydrocarbons and heavy metals in Alaska arctic marine mammals. Mar. Pollut. Bull. 40, 819-829.

Becker, P.R., Wise, S.A., 2006. The U.S. National Biomonitoring Specimen Bank and the Marine Environmental Specimen Bank. J. Environ. Monit. 8, 795-799.

Beckmen, K.B., Duffy, L.K., Zhang, X.M., Pitcher, K.W., 2002. Mercury concentrations in the fur of Steller sea lions and northern fur seals from Alaska. Mar. Pollut. Bull. 44, 1130-1135.

Bell, L.S., Cox, G., Sealy, J., 2001. Determining isotopic life history trajectories using bone density fractionation and stable isotope measurements: a new approach. Am. J. Phys. Anthropol. 116, 66-79.

Bilandzic, N., Dezdek, D., Sedak, M., Dokic, M., Solomun, B., Varenina, I., Knezevic, Z., Slavica, A., 2010. Concentrations of trace elements in tissues of red fox (*Vulpes vulpes*) and stone marten (*Martes foina*) from suburban and rural areas in Croatia. Bull. Environ. Contam. Toxicol. 85, 486-491.

Boundless, Jul. 03 2014. Internal anatomy of the kidney. Boundless Anatomy and PhysiologyBoundless (Retrieved 19 Feb. 2015 from https://www.boundless.com/ physiology/textbooks/boundless-anatomy-and-physiology-textbook/the-urinary- system-25/kidneys-239/internal-anatomy-of-the-kidney-1168-4690/).

Brookens, T.J., O'Hara, T.M., Taylor, R.J., Bratton, G.R., Harvey, J.T., 2008. Total mercury body burden in Pacific harbor seal, *Phoca vitulina richardii*, pups from central California. Mar. Pollut. Bull. 56, 27-41.

Castellini, J., Rea, L., Lieske, C., Beckmen, K., Fadely, B., Maniscalco, J., O'Hara, T., 2012. Mercury concentrations in hair from neonatal and juvenile Steller sea lions (*Eumetopias jubatus*): implications based on age and region in this northern pacific marine sentinel piscivore. EcoHealth 9 (3), 267-277.

Castoldi, A.F., Coccini, T., Cecatelli, S., Manzo, L., 2001. Neurotoxicity and molecular effects of methylmercury. Brain Res. Bull. 55, 197-203.

Cavallini, P., Lovari, S., 1991. Environmental factors influencing the use of habitat in the red fox, *Vulpes vulpes*. J. Zool. (Lond.) 223, 323.

Cernichiari, E., Brewer, R., Myers, G.J., Marsh, D.O., Lapham, L.W., Cox, C., Shamlaye, C.F., Berlin, M., Davidson, P.W., Clarkson, T.W., 1995. Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. Neurotoxicology 16, 705-710.

Ciampalini, B., Lovari, S., 1985. Food habits and trophic niche overlap of the badger *Meles meles* L. and the red fox *Vulpes vulpes* L. in a Mediterranean coastal area. Z. Säugetierkd. 50, 226.

Clarkson, T.W., 1997. The toxicology of mercury. Crit. Rev. Clin. Lab. Sci. 34, 369-403.

Clarkson, T.W., Magos, L., 2006. The Toxicology of Mercury and Its Chemcial Compounds. Crit. Rev. Toxicol. 36, 609-662.

Corsolini, S., Focardi, S., Leonzio, C., Lovari, S., Monaci, F., Romeo, G., 1999. Heavy metals and chlorinated hydrocarbon concentrations in the red fox in relations to some biological parameters. Environ. Monit. Assess. 54, 87-100.

Cumbie, P.M., 1975. Mercury in hair of bobcats and raccoons. J. Wildl. Manag. 39, 419-425.

Cumbie, P.M., Jenkins, J.H., 1974. Mercury Accumulation in Native Mammals of the Southeast. In: SG&F Commissioners (Ed.), White Sulfur Springs, West Virgina.

Cybulski, W., Chalabis-Mazurek, A., Jakubczak, A., Jarosz, L., Kostro, K., Kursa, K., 2009. Content of lead, cadmium, and mercury in the liver and kidneys of silver foxes (*Vulpes vulpes*) in relation to age and reproduction disorders. Bull. Vet. Inst. Pulawy 53, 65-69.

Davis, L.E., Kornfield, M., Mooney, H.S., Fiedler, K.J., Haaland, K.Y., Orrison, W.W., Cermichiari, E., Clarkson, T.W., 1994. Methylmercury poisoning: long-term clinical, radiological, toxological, and pathological studies of an affected family. Annu. Neurol. 35, 680-688.

Dehn, L.A., Follmann, E.H., Thomas, D.L., Sheffield, G.G., Rosa, C., Duffy, L.K., O'Hara, T.M., 2006. Trophic relationships in an Arctic food web and implications for trace metal transfer. Sci. Total Environ. 362, 103-123.

Dell'Arte, G.L., Laaksonen, T., Norrdahl, K., Korpimaki, E., 2007. Variation in the diet composition of a generalist predator, the red fox, in relation to season and density of main prey. Acta Oecol. 31, 276-281.

Dietz, R., Outridge, P.M., Hobson, K.A., 2009. Anthropogenic contributions to mercury levels in present-day Arctic animals—a review. Sci. Total Environ. 407, 6120-6131.

Dietz, R., Born, E.W., Riget, F., Aubail, A., Sonne, C., Drimmie, R., Basu, N., 2011. Temporal trends and future predictions of mercury concentrations in Northwest Greenland polar bear (*Ursula maritimus*) hair. Environ. Sci. Technol. 45, 1458-1465.

Dip, R., Stieger, C., Deplazes, P., Hegglin, D., Muller, U., Dafflon, O., Koch, H., Naegeli, H., 2001. Comparison of heavy metal concentrations in tissues of red foxes from adjacent urban, suburban, and rural areas. Arch. Environ. Contam. Toxicol. 40, 551-556.

Doncaster, C.P., Macdonald, D.W., 1991. Ecology and ranging behavior of red foxes in the city of Oxford. Hysteix 3, 11.

Doyle, J.J., 1979. Toxic and essential elements in bone — a review. J. Anim. Sci. 49, 482-497.

Duffy, L.K., Duffy, R.S., Finstad, G.L., Gerlach, C., 2005. A note on mercury levels in the hair of Alaskan reindeer. Sci. Total Environ. 339, 273-276.

Dunlap, K.L., Reynolds, A.J., Bowers, P.M., Duffy, L.K., 2007. Hair analysis in sled dogs (*Canis lupus familiaris*) illustrates a linkage of mercury exposure along the Yukon River with human subsistence food systems. Sci. Total Environ. 385, 80-85.

Dwek, J., 2010. The periosteum: what is it, where is it, and what mimics it in its absence? Skelet. Radiol. 39, 319-323.

Gaden, A., Ferguson, S.H., Harwood, L., Melling, H., Stern, G.A., 2009. Mercury trends in ringed seals (*Phoca hispida*) from the Western Canadian Arctic since 1973: associations with length of ice-free season. Environ. Sci. Technol. 43, 3646-3651.

Gamberg, M., Boila, G., Stern, G., Roach, P., 2005. Cadmium, mercury, and selenium concentrations in mink (*Mustela vison*) from Yukon, Canada. Sci. Total Environ. 351, 523-529.

Goldyn, B., Hromada, M., Surmachi, A., Tryjanowski, P., 2003. Habitat use and diet of the red fox *Vulpes vulpes* in an agricultural landscape in Poland. Z. Jagdwiss. 49, 191-200.

Gutleb, A.C., Kranz, A., Nechay, G., Toman, A., 1998. Heavy Metal Concentrations in Livers and Kidneys of the Otter (*Lutra lutra*) from Central Europe. Bull. Environ. Contam. Toxicol. 60, 273-279.

Halffman, C., 2009. Bone as a biomarker of mercury exposure in prehistoric arctic human populations: initial method validation using animal models. (PhD Dissertation), University of Alaska Fairbanks, Fairbanks, AK.

Hersteinsson, P., Macdonald, D.W., 1992. Interspecific competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus*. Oikos 64, 505-515.

Hewson, R., Kolb, H.H., 1975. The food of foxes (*Vulpes vulpes*) in Scottish forests. J. Zool. (Lond.) 176, 287.

Jones, D.M., Theberge, J.B., 1982. Summer home range and habitat utilization of the red fox (*Vulpes vulpes*) in a tundra habitat, northwest British Columbia. Can. J. Zool. 60, 807-812.

Kalisinska, E., Lisowski, P., Salicki, W., Kucharska, T., Kavetska, K., 2009. Mercury in wild terrestrial carnivorous mammals from north-western Poland and unusual fish diet of red fox. Acta Theriol. 54, 345-356.

Kalisinska, E., Lisowski, P., Kosik-Bogacka, D.I., 2012. Red fox *Vulpes vulpes* (L., 1758) as a bioindicator of mercury contamination in terrestrial ecosystems of north-western Poland. Biol. Trace Elem. Res. 145, 172-180.

Kamler, J.F., Ballard, W.B., 2002. A review of native and nonnative red foxes in North America. Wildl. Soc. Bull. 30, 370-379.

Kiener, T.V., Zaitsev, V.A., 2010. Range structure in the red fox (*Vulpes vulpes* L.) in the forest zone of Eastern Europe. Contemp. Probl. Ecol. 3, 119-126.

Klepinger, L.L., 1984. Nutritional assessment from bone. Annu. Rev. Anthropol. 13, 75-96.

Knott, K.K., Schenk, P., Beyerlein, S., Boyd, D., Ylitalo, G.M., O'Hara, T.M., 2011a. Blood- based biomarkers of selenium and thyroid status indicate possible adverse biological effects of mercury and polychlorinated biphenyls in Southern Beauford Sea polar bears. Environ. Res. 111, 1124-1136.

Knott, K.K., Boyd, D., Ylitalo, G.M., O'Hara, T.M., 2011b. Concentrations of mercury and polychlorinated biphenyls in blood of Southern Beaufort Sea polar bears (*Ursula maritimus*) during spring: variations with lipids and stable isotope ($\delta^{15}N$, $\delta^{13}C$) values. Can. J. Zool. 89, 999-1012.

Kwapulinski, J., Miroslawski, J., Wiechula, D., Jurkiewicz, A., Tokarowski, A., 1995. The femur capitulum as a biomarker of contamination due to indicating lead content in the air by participation of the other metals. Sci. Total Environ. 175, 57-64.

Lanocha, N., Kalisinska, E., Kosik-Bogacka, D.I., Budis, H., Noga-Deren, K., 2012. Trace metals and micronutrients in bone tissues of the red fox *Vulpes vulpes* (L., 1758). Acta Theriol. 57, 233-244.

Lanocha, N.E., Kalisinska, E., Kosik-Bogacka, D.I., Budis, H., Podiasinski, J., Jedrzejewskia, E., 2014. Mercury levels in raccoons (*Procyon lotor*) from the Warta Mouth National Park, northwestern Poland. Biol. Trace Elem. Res. 159 (1-3), 152-160.

Lehner, N., 2012. Arctic Fox Winter Movement and Diet in Relation to Industrial Development on Alaska's North Slope. (MS Thesis). University of Alaska Fairbanks, Fairbanks, AK.

Lieske, C.L., Moses, S.K., Castellini, J.M., Klejka, J., Hueffer, K., O'Hara, T., 2011. Toxicokinetics of mercury in blood compartments and hair of fish-fed sled dogs. Acta Vet. Scand. 53, 1-9.

Lokken, J.A., Finstad, G.L., Dunlap, K.L., Duffy, L.K., 2009. Mercury in lichens and reindeer hair from Alaska: 2005-2007 pilot survey. Polar Rec. 45 (235), 368-374.

Lopez-Artiguez, M., Camean, A., Gonzalez, G., Repetto, M., 1995. Metal accumulation in human kidney cortex: mutual interrelations and effect of human factors. Hum. Exp. Toxicol. 14, 335-340.

Lovari, S., Valier, P., Ricci Lucchi, M., 1994. Ranging behaviour and activity of red foxes (*Vulpes vulpes*: Mammalia) in relation to environmental variables, in a Mediterranean mixed pinewood. J. Zool. (Lond.) 232, 323.

Martinez-Garcia, M.J., Moreno, J.M., Moreno-Clavel, M., Vergara, N., Garcia-Sanchez, A., Guillamon, A., Porti, M., Moreno-Grau, S., 2005. Heavy metals in human bones in different historical epochs. Sci. Total Environ. 348, 51-72.

McGrew, A., Ballweber, L., Moses, S., Stricker, C., Beckmen, K., Salman, M., O'Hara, T., 2013. Mercury in gray wolves (*Canis lupus*) in Alaska: increased exposure through consumption of marine prey. J. Total Environ. 468-469, 609-613.

McIntosh, D.L., 1963. Food of the red fox in the Canberra district. Wildl. Res. 8, 1.

Millan, J., Mateo, R., Taggart, M.A., Lopez-Bao, J.V., Viota, M., Monsalve, L., Camearero, P.R., Blazquez, E., Jimenez, B., 2008. Levels of heavy metals and metalloids in critically endangered Iberian lynx and other wild carnivores from Southern Spain. Sci. Total Environ. 399, 193-201.

Nielsen-Marsh, C.M., Hedges, R.E.M., 1999. Bone porosity and the use of mercury intrusion porosimetry in bone diagenesis studies. Archaeometry 41, 165-174.

O'Hara, T., Hanns, C., Woshner, V.M., Bratton, G., Taylor, R., 2008. Essential and non-essential elements in the bowhead whale: epidermis-based predictions of blubber, kidney, liver and muscle tissue concentrations. J. Cetacean Res. Manag. 10 (2), 107-117.

Piskorová, L., Vasilková, Z., Krupicer, I., 2003. Heavy metal residues in tissues of wild boar (*Sus scrofa*) and red fox (*Vulpes vulpes*) in the Central Zemplin region of the Slovak Republic. Czech J. Anim. Sci. 48, 134-138.

R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria 3-900051-07-0 (Retrieved 20 Jan. 2012 from http://www.R-project.org).

Rytuba, J.J., 2003. Mercury from mineral deposits and potential environmental impact. Environ. Geol. 43, 326-338.

Saunders, G., White, C.L., Harris, S., Rayner, M.V., 1993. Urban foxes (*Vulpes vulpes*): food acquisition, time and energy budgeting of a generalized predator. Symp. Zool. Soc. (Lond.) 65, 215-234.

Schutkowski, H., Herrmann, B., 1999. Diet, status and decomposition at Weingarten: trace element and isotope analyses on early mediaeval skeletal material. J. Archaeol. Sci. 26, 675-685.

Takata, M.K., Saiki, M., 2004. INAA of cortical and trabecular bone samples from animals. J. Radioanal. Nucl. Chem. 259, 375-379.

von Schantz, T., 1980. Prey consumption of a red fox population in Southern Sweden. Biogeographica 18, 53.

Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H., Scheuhammer, A.M., 2003. Ecotoxicology of Mercury. In: Hoffman, D.J., Rattner, B.A., Burton Jr., G.A., Cairns Jr., J. (Eds.), Lewis Publishers, Boca Raton, FL.

Wolfe, M.F., Schwarzbach, S., Sulaiman, R.A., 1998. Effects of mercury on wildlife: a comprehensive review. Environ. Toxicol. Chem. 17, 146-160.

Wren, C.D., 1986. A review of metal accumulation and toxicity in wild mammals: I. Mercury. Environ. Res. 40, 210-244.

Yamamoto, Y., Honda, K., Hidaka, H., Tatsukawa, R., 1987. Tissue distribution of heavy metals in Weddell seas (*Leptonychotes weddellii*). Mar. Pollut. Bull. 18, 164-169.

Yin, X., Sun, L., Zhu, R., Liu, X., Ruan, D., Wang, Y., 2007. Mercury–selenium association in antarctic seal hairs and animal excrements over the past 1,500 years. Environ. Toxicol. Chem. 26, 381-386.

Zwanziger, H., 1989. The multielemental analysis of bone. Biol. Trace Elem. Res. 19, 195-232.

1.9 Figures

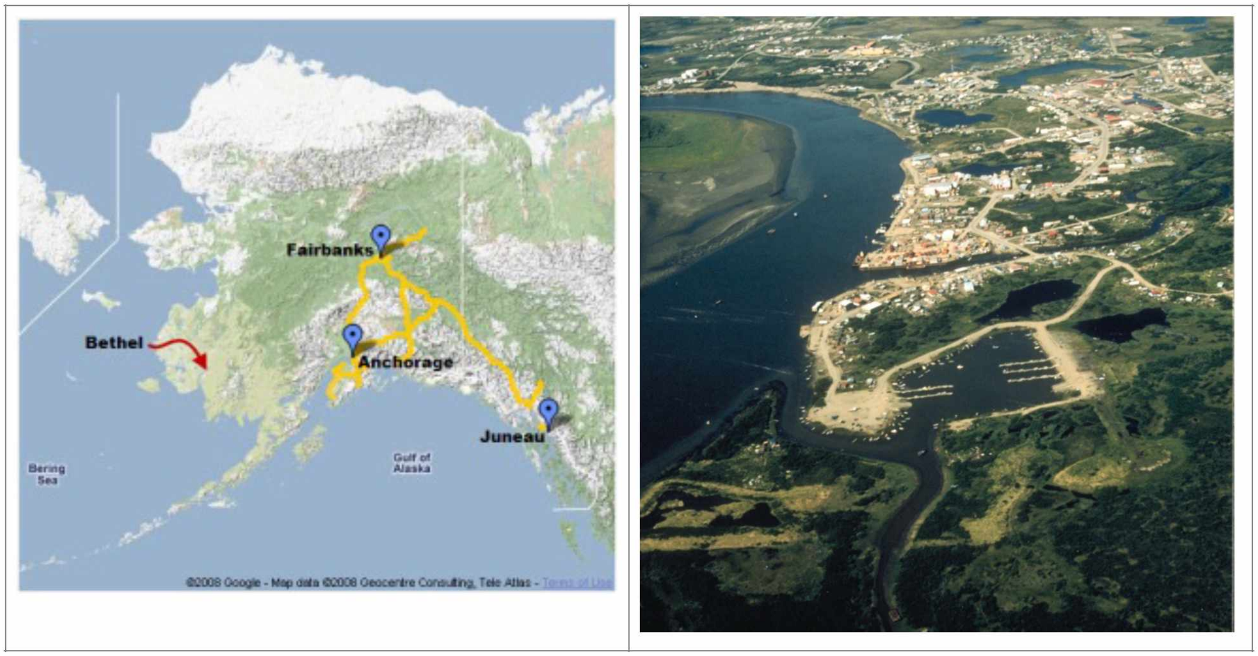

Figure 1.1: Bethel Alaska and the Kuskokwim River.

Fig. 1.2: Showing where cores were drilled through femur.

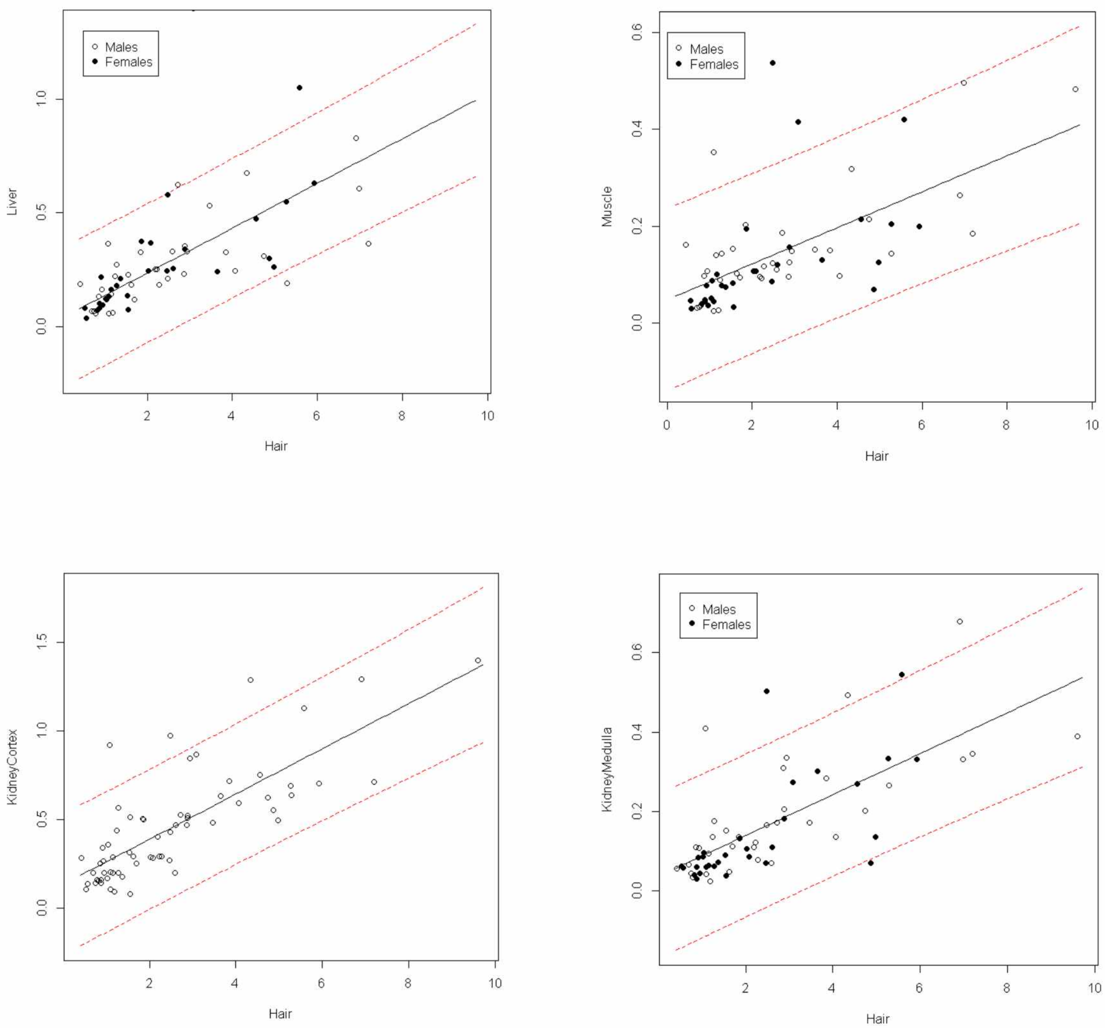

Figure 1.3: Fitted regression models for hair THg mg/kg (x-axis) vs each soft tissue THg mg/kg (y-axis). Models fitted with 95% pointwise prediction intervals. Clockwise from top left: hair vs. liver, hair vs. muscle, hair vs. renal medulla, hair vs. renal cortex.

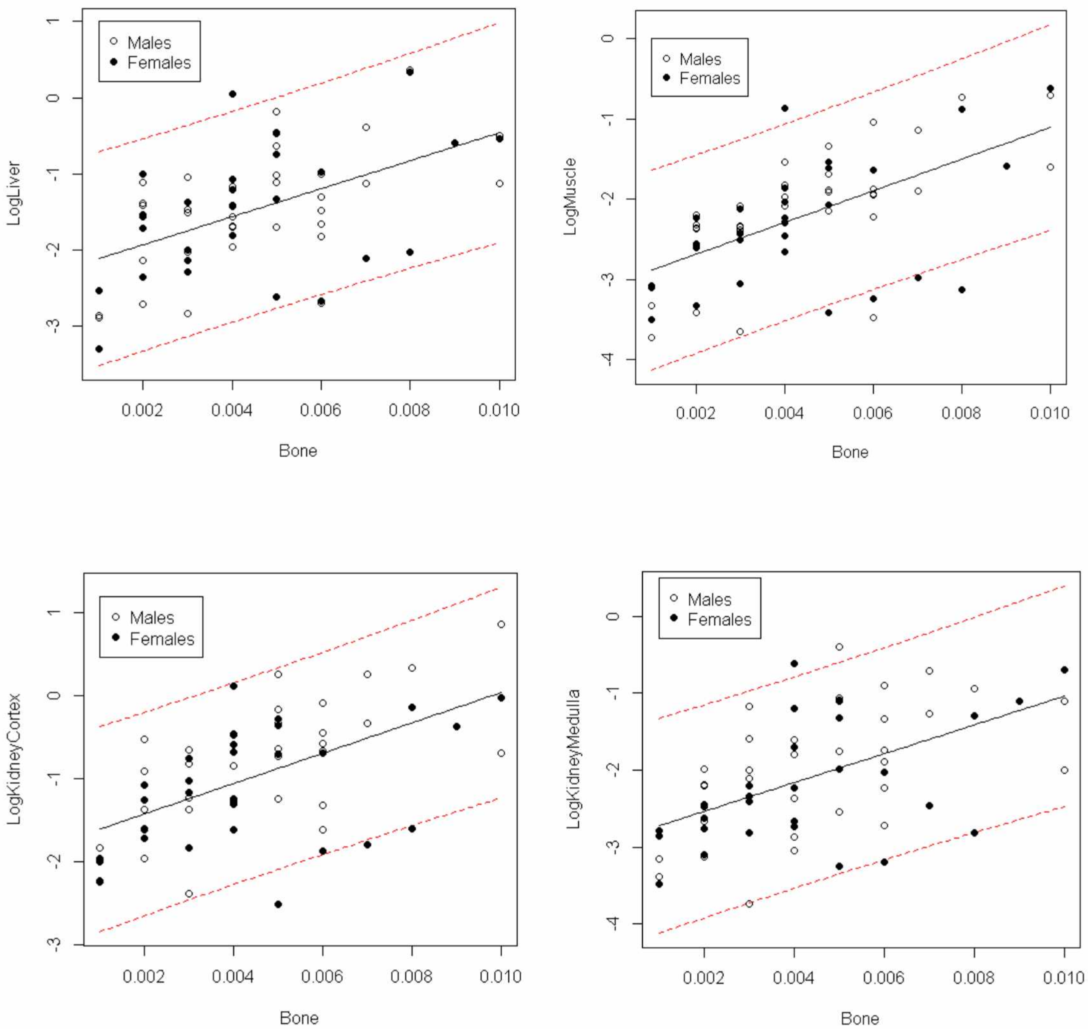

Fig. 1.4: Regression models with bone THg mg/kg (x-axis) vs each soft tissue THg mg/kg (y-axis). Models fitted with 95% pointwise prediction intervals. Clockwise from top left: bone vs. liver, bone vs. muscle, bone vs. renal medulla, bone vs. renal cortex.

1.10 Tables

Table 1.1: Percent recovery of liquid standards and certified reference materials

	% Recovery				
	Bone	Hair	Liver	Renal Cortex & Medulla	Muscle
Liquid Standard[a] (µg/g)					
0.010	0.010				
0.100		0.097	0.099	0.096	0.103
1.000		0.987		0.964	1.043
Reference Materials (g)					
SRM 1486[b](0.0300)	109.75				
IAEA 085[c] (0.0200)		101.43			
IAEA 086[c] (0.0200)		106.32			
DORM3[d] (0.0100)			107.95	102.60	110.07
DOLT4[e] (0.0100)			110.50	97.83	109.62

[a]Liquid Standards: 0.010 in 3.7% HCL [9.913 ppb] for bone; 0.100 in 3.7% HCL [99.9 ppb], 1.000 in 3.7% HCL [.999 ppm] for hair and renal cortex/medulla; 0.100 in 3.7% HCL [100.52 ppb] for liver; 0.100 in 3.7% HCL [99.02] for muscle.

[b] Bone meal SRM 1486 (note: Author worked with Dr. Stephen Long of the National Institute of Standards and Technology. This SRM will now be assigned a reference value on the certificate)

[c] Hair certified standard; IAEA 085 = 23.2 µg/g; IAEA 086 = 0.573 µg/g; International Atomic Energy Agency

[d] Fish protein certified standard; 0.382 ± 0.060 µg/g; National Research Council of Canada

[e] Dogfish liver certified standard; 2.54 ± 0.22 µg/g; National Research Council of Canada

Table 1.2: Mean±SD, minimum, maximum, and median mercury concentration, for each tissue type combining male and female red fox and separated by sex.

Tissue	Mean ± SD	Minimum	Maximum	Median
Bone	0.004 ± 0.002	0.001	0.010	0.004
Hair	2.580 ± 1.959	0.429	9.604	2.024
Muscle	0.567 ± 0.464	0.097	2.001	0.432
Liver	1.226 ± 1.133	0.114	6.149	0.924
Renal Medulla	1.177 ± 0.968	0.217	4.061	0.754
Renal Cortex	2.122 ± 1.486	0.429	6.049	1.929
Bone				
Male	0.005 ± 0.002	0.001	0.010	0.004
Female	0.004 ± 0.002	0.001	0.010	0.004
Hair				
Male	2.794 ± 2.188	0.429	9.604	2.222
Female	2.320 ± 1.666	0.539	5.926	1.705
Muscle				
Male	0.608 ± 0.441	0.097	1.932	0.510
Female	0.519 ± 0.494	0.114	2.001	0.341
Liver				
Male	1.261 ± 1.101	0.225	6.149	0.969
Female	1.185 ± 1.188	0.114	5.334	0.887
Renal Medulla				
Male	1.307 ± 0.968	0.217	3.794	0.996
Female	1.026 ± 0.961	0.256	4.061	0.632
Renal Cortex				
Male	2.341 ± 1.575	0.429	6.049	2.037
Female	1.867 ± 1.356	0.509	5.621	1.340

Reported in mg/kg dw

Table 1.3: R^2 and p-values for testing association between hair and bone vs soft tissues (no sex or interaction)*.

		Renal Cortex	Renal Medulla	Liver	Muscle
Hair	R^2	0.6073	0.5003	0.6268	0.3886
	p-value	3.34e-14	4.5-7e-11	6.804e-15	2.383e-07
Bone	R^2	0.3287	0.2868	0.2768	0.3595
	p-value	5.997e-07	4.293e-06	6.76e-05	1.31e-07

* Responses for bone were log transformed

Chapter 2 A Stable Carbon and Nitrogen Isotope Investigation of Tissues in the Free-Ranging Western Alaska Red Fox[1]

2.1 Abstract

Stable carbon and nitrogen isotopes in tissues from one population of wild free-ranging red foxes (*Vulpes vulpes*) in Western Alaska were used to 1) examine the lipid extraction process, 2) evaluate carbon and nitrogen correlations among tissues to establish stable isotope values for modern northern wild fox populations, 3) describe the C:N ratios in males and females, 4) establish trophic positions of free-ranging northern red foxes, and 5) relate $\delta^{15}N$ values to mercury biomagnification. Our results showed that hair stable isotopes are correlated with other tissue stable isotope values. Results also showed that hair, bone, muscle, liver, renal cortex and medulla tissues of the red fox were isotopically significantly different from each other. We found that the Western Alaska red fox was likely eating a different diet based upon a lower trophic position than red foxes from other northern areas. We concluded that stable isotope data can help explain mercury concentration levels influenced by seasonal diet changes of the Alaska red fox.

---

[1] Dainowski BH, Duffy LK, McIntyre J. 2016. A Stable Carbon and Nitrogen Isotope Investigation of Various Tissues in the Free-Ranging Western Alaska Red Fox. Prepared for submission to Science of the Total Environment.

2.2 Introduction

The red fox's (*Vulpes vulpes*) range expanded into the Arctic regions of Canada and Eurasia during the 20th century (Chirkova, 1968; Hersteinsson and Macdonald, 1992; Kiener and Zaitsev, 2010). Their populations increased first in the foothills and river corridors of the Arctic Coastal Plain (Bee and Hall, 1956; Brower, 1942). Red foxes are considered generalist omnivore predators (Dainowski et al., 2015; Larivière and Pasitschniak-Arts, 1996; Newsome et al., 2014; Tesky, 1995), whose movements are usually driven by local and seasonal food availability.

Food sources for red foxes often consist of rodents, invertebrates, fruit, and even garbage in small urban settings (Savory et al., 2014; Von Schantz, 1980). Red foxes can survive in a variety of ecosystems from semi-arid deserts to the tundra (Dell'Arte et al., 2007; Lindström, 1983; Sargeant, 1978), feeding on a wide range of prey species specific to their habitat (Harris, 1981; Roth and Hobson, 2000; Savory, 2013). In western Alaska, the natural habitat contains many different types of prey for the red fox, including wood frogs (*Rana sylvatica)*, (Bergeron et al., 2011; Reeves and Trust, 2008), lemmings (*Lemmus sibiricus* and *Dicrostonyx torquatus)*, and other microtine rodents, shrews (*Sorex cinereus)* (Gotthardt and McClory, 2006), black fish (*Dalia pectoralice)* (Armstrong, 1994), and birds, such as willow ptarmigans (*Lagous lagopus)* (Kelly, 2000; Peterson and Fry, 1987).

Stable isotope studies traditionally trace pathways of organic matter using $\delta^{13}C$ and $\delta^{15}N$ (McCutchan et al., 2003). Different stable isotope ratios ($^{13}C/^{12}C$) also arise in the photosynthetic pathways of C_3 and C_4 plants (Farquhar et al., 1989; Smith and Epstein, 1971; Vogel and Van Der Merwe, 1977). Here in Western and Arctic Alaska, the native vegetation is exclusively composed of C_3 plants (Sage et al.,1999). Stable carbon isotopes are reflective of naturally occurring isotope values in animals' diets and reflective of animals' movement patterns (Dalerum and Angerbjörn, 2005; Lehner, 2012; Peterson and Fry, 1987). This has been established by stable isotope ratios reflecting dietary sources from coastal, terrestrial, benthic and pelagic environments (France, 1995; Hobson et al., 2002; Rau et al., 1992; Rounick and Winterbourn, 1986); distinguishing Arctic wild foods from processed store-bought foods, because of the difference in carbon isotopes (Fry, 2006; Jahren and Kraft, 2008; Jahren et al., 2006; Lehner, 2012).

The stable nitrogen isotope ($^{15}N/^{14}N$) has been used to identify food web structures (Bocherens and Drucker, 2003; Minagawa and Wada, 1984; Roth and Hobson, 2000; Schoeninger and DeNiro, 1984). Nitrogen isotope values are also used to establish trophic levels (Dehn et al., 2006; DeNiro and Epstein, 1981; Fry, 2006; Post, 2002).

Complicated issues can arise using stable isotope signatures in dietary studies. One such issue is the process by which isotopes are incorporated into the animals' tissues. Isotopic signatures are influenced by an animals' age, nutritional status, size, tissue type, and the macronutrients in the diet (Ben-David and Flaherty, 2012; Bond and Diamond, 2010; Fry, 2006; Hobson and Quirk, 2014; Kelly, 2000; Robbins et al., 2005). Besides isotopic incorporation, these factors also discriminate between the animals' diet and its tissues (Ben-David et al., 2012). For example, the $\delta^{15}N$ signature will be incorporated in the tissues at a slow rate when herbivores consume a nitrogen-poor diet because they will be using the nitrogen storage in their bodies (Ben-David and Flaherty, 2012). In addition, an animals feeding ecology can be further complicated by the uptake of contaminants, such as mercury, through their food webs (Kalisinska et al., 2009).

Several other important considerations arise in dietary studies using stable isotopes. First, lipid extraction is a correction commonly applied in stable isotope studies on marine mammals to reduce variability and bias introduced when tissues have high lipid content. This correction may also be important for terrestrial animals feeding in coastal regions (Ben-David et al., 2012) due to fish consumption.

In addition, each tissue incorporates the nutrients from diet differently. It is important to understand how different tissue $\delta^{13}C$ values are related to each other, as each tissue is influenced by different turnover rates (Carroll et al., 2013; Kielland, 2001; Murray et al., 2015). Besides turnover rate, it is important to understand if there is any intra-specific stable isotope values from one tissue compared to other tissues of the same species, to see if the consistency is similar across males and females, as this may provide insight into ecological drivers of dietary variations (Sweeting et al., 2005). It is also important to see which tissue is more suitable to use in a diet study in order to determine feeding and migration habits. For example, soft tissues such as muscle, kidney and liver may reflect stable isotopes differently from each other, and perhaps quite differently from hard tissues such as hair and bone, leading to tissue-dependent discrepancies in estimates of diet composition. Due to different metabolic turnover rates, stable isotopes in different tissues can represent diet over different time periods (Phillips and Eldridge, 2006). Therefore, stable isotope analyses of winter hair of foxes can infer a late summer diet, because the winter hair is grown in September and October, prior to the start of winter (Chesemore, 1967; Maurel et al., 1986; Roth and Hobson, 2000). On the other hand, muscle tissue can infer a diet one to two months prior to sampling (Roth and Hobson, 2000; Lecomte et al., 2011). Bone collagen has a slower turnover period (Tieszen et al., 1983) and is used to infer a lifetime diet of the red fox.

Another important consideration is the dependence of isotope values on factors such as sex. Understanding the relationships among isotope measurements on both sexes and on a variety of tissues will allow better interpretation and comparison of different studies in the literature. Moreover, it may also facilitate comparison of the diets of modern animals to diets inferred from historical museum specimens where only hair or bone is available and the sex of the animal is unknown.

Lastly, current climate changes affect ecosystems by forcing species to migrate into different habitats and can therefore affect the prey availability (Lovejoy and Hannah, 2006). As the Arctic's climate changes, (Murray et al., 2015) the prey of the red fox only becomes harder to find, leading to changes in diet and trophic level. Establishing current feeding patterns is important for the long-term monitoring of this sentinel species.

In this study we use stable isotopes to investigate diet characteristics of the red fox using foxes sampled from Bethel, Alaska. We first consider the necessity of lipid extraction on the tissues of the sampled foxes. Next we compare mean values of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), as well as the carbon-to-nitrogen ratio (C:N) between male and female foxes for several soft and hard tissues (kidney cortex, kidney medulla, liver, muscle, hair and bone). We similarly compare mean isotope values among all pairs of tissues, and measure the correlations in isotope values between all pairs of tissues. Finally we use our results to infer diet characteristics of red foxes in Northwest Alaska and discuss them in the context of other published diet studies on foxes. Additionally, the potential use of stable isotope information to infer bioaccumulation of contaminants such as total mercury is discussed.

2.3 Materials and Methods

2.3.1 *Sample preparation and stable isotope analysis*

A total of 200 red fox carcasses were donated during the period of November 2010 through February 2011. Trappers anonymously and voluntarily provided carcasses to the Alaska Department of Fish and Game (ADF&G) in Bethel, Alaska. Carcasses remained frozen and were stored outside at the ADF&G office (temperature was continuously below 0°C).

Frozen carcasses were transported to the University of Alaska Fairbanks (UAF) where they were stored at -20˚C. Carcasses were partially thawed and sub-samples of liver, muscle, and one whole kidney, femur, and rear paw with fur were collected into Whirl-Pak™ bags and stored at -20˚C (liver, muscle, kidney, rear paw) and -80°C (femur). All samples were collected from the right side of the carcass using stainless steel scalpels or scissors. Only foxes with all five available tissues were analyzed for stable isotope analysis. Based on these criteria, 65 foxes were used in this study, 35 males and 30 females.

Approximately 14 g of frozen liver was cut into small pieces (~1 cm^3). The entire quadricep muscle was removed from the femur bone while partially frozen, and cut into small pieces. For dissection of cortex and medulla, kidneys were kept partially frozen on a clean stainless steel tray placed on ice. All instruments used to separate renal cortex and medulla (scalpels and trays) were also kept at -20°C before use. All tissues were placed into individual pre-weighed Whirl-Pak™ bags and lyophilized for 72 hours (Labconco FreeZone 4.5™ Freeze Dry System).

Hair was collected from the right rear paw using a Wahl stainless steel trimmer (carbon blades) and stainless steel scissors. Blades were thoroughly cleaned between samples. Hair was placed in a 50 ml metal-free conical bottom tube and filled with 1% Triton X, covered, and allowed to sit for 15-30 minutes, shaking occasionally. The Triton X was then poured off and the tube was filled with H_2O, covered, sat for 10-15 minutes, with occasional shaking. This process was repeated at least four times until all soap was gone. The hair samples were placed into individual pre-weighed Whirl-Pak™ bags and lyophilized for 48 hours (Labconco FreeZone 4.5™ Freeze Dry System).

Bones were cored under a fume hood while wearing a 3M™ particulate respirator N95. The bones were cored using a Dremel™ glass diamond drilling bit, ¼" (6.350mm), 663DR, drill speed #23. Each femur was drilled completely through the shaft in three locations to produce six core. Four cores were used in a similar study of THg (Dainowski et al., 2015), and the remaining 2 cores were used in this stable isotope study. The periosteum and any traebecular bone were removed using a Dremel™ glass diamond taper point sander, 3/32" (2.381mm), #7144. The cores were stored at -20°C in acid-washed (5% HNO_3) vials prior to degreasing. The degreasing method used is as follows: bone core samples were sonicated in deionized water in order to remove any debris. Next, they were soaked in chloroform for two 8 hour periods to remove any lipids, then finally rinsed with deionized water and air-dried under a fume hood for 3 days.

The bone collagen extraction was a variation of the methods set forth by Matheus (1997). The method is as follows: bone samples were demineralized by soaking in an HC1-H_2O solution until they were translucent. After which they were rinsed with deionized water. In order to separate the collagen from other organic compounds and proteins, the bone samples were gelatinized by placing the collagen samples, with HC1-H_2O solution (pH between 3.0 and 4.0) in a culture tube. The tube was heated to 70°C under a stream of nitrogen, to dissolve the collagen. The supernatant was lyophilized to retrieve the collagen after centrifugation.

Isotope ratios are presented as stable isotope abundances relative to the international standard (delta (δ) notation) in parts per thousand (permil (‰):

$$\delta X = ((Rsample/Rstandard)-1) \times 10^3, ‰$$

where, element X = ^{13}C or ^{15}N and R is the molar ratios of heavy to light isotope of the sample and the standard (Fry, 2006). These isotope ratios are expressed in delta notation of per mil relative to the Viennna PeeDee Belemnite international standards for carbon, and atmospheric N for nitrogen. These standard values are measured using a laboratory standard (peptone) calibrated against several certified reference materials (Fry, 2006).

Between 0.1 and 0.5 mg of hair, liver, muscle, renal cortex, renal medulla, and bone collagen samples were placed in tin capsules then loaded into an autosampler. Samples were analyzed for stable carbon and nitrogen isotopes at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks. A continuous-flow isotope ratio mass spectrometry with a Costech ECS4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the Conflo III interface (Thermo Fisher Scientific, Waltham, MA, USA) was used for analyzing. The laboratory standard was measured multiple times between samples to evaluate the accuracy and precision of analyses. Accuracy and precision was assessed using multiple peptone standards. Precision was within 0.3‰ and accuracy was within 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

2.3.2 *Tissue turnover, growth rates, and lipid contamination methods used for analysis*

Analysis of the pelage cycle (hair) of the red fox has shown that hair loss occurs in late spring, approximately in May, and regrowth occurs late summer through October. Therefore, the hair collected in winter will be representative of a diet August through October (Maurel et al., 1986). Bone collagen, however, describes the lifetime diet of an animal (Tieszen et al., 1983), while muscle tissues will show a diet within the last two months prior to sampling (Roth and Hobson, 2000). Renal cortex and medulla, as well as the liver show the diet within the last week prior to sampling (Roth and Hobson, 2000; Roth et al., 2007).

Just like corn (C_4) or wheat (C_3) diets, lipids have been found to be another secondary fractionation issue in some stable isotope studies. This is because lipids are typically depleted in ^{13}C and have $\delta^{13}C$ values more negative than those in carbohydrates and proteins within an individual organism (DeNiro and Epstein, 1977; Post et al., 2007). Lipids for hair and bone were extracted, while muscle, liver and kidney were not. The C:N ratios were analyzed to compare the effect of lipid extractions. A C:N ratio of around four (~10% lipids) is an acceptable range for terrestrial mammals.

2.3.3 *Statistical analysis*

Histograms and normal probability plots were used to assess normality of the distribution of $\delta^{13}C$ and $\delta^{15}N$ isotope measurements for each tissue. Individual isotope measurements greater than three standard deviations from the mean for each tissue were identified as potential outliers (Ruan et al., 2005). Means and standard deviations were used to summarize $\delta^{13}C$, $\delta^{15}N$ and C:N values for all tissues for all foxes, as well as separately for male and female foxes. T-tests were used to compare mean isotope values of males and females for each tissue. Paired *t*-tests were used to test mean differences in isotope values between pairs of tissues, for both $\delta^{13}C$ and $\delta^{15}N$. Pearson's product-moment correlation was used to measure and test the significance of correlations between isotope values in each pair of tissues, for both $\delta^{13}C$ and $\delta^{15}N$. We used the scale established by Andale (2015) to classify correlations as follows: High correlation: 0.5 to 1.0 or -0.5 to 1.0, Medium correlation: 0.3 to 0.5 or -0.3 to 0.5, and Low correlation: 0.1 to 0.3 or -0.1 to -0.3. A significance level of α =.05 was used for all hypothesis tests. All analyses were performed using the statistical software R (R Development Core Team, 2015).

2.4 Results

2.4.1 *Isotope values for* $\delta^{13}C$ and $\delta^{15}N$ *and C:N ratios in wild foxes*

The small percentage of lipid content found in a few tissues did not influence the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values. These red fox tissues had a 1% difference in their mean values between lipid extracted, e.g. bone, and non-lipid extracted tissues, e.g. hair for $\delta^{13}C$. The C:N ratios demonstrated a ratio that would be expected for terrestrial mammals (Post et al., 2007). As a result, no correction for lipids were performed on the unextracted samples (Fagan et al., 2011; Post et al., 2007).

Histograms and normal probability plots of isotope values were consistent with normality for all tissues. An outlier was identified in one fox that had isotope values outside the three standard deviation range for $\delta^{13}C$ in hair and bone, as well as $\delta^{15}N$ in hair, bone and muscle (circled points in Figure 2.1). This same fox was also considerably larger and heavier (5.5 kilograms (kg)) than all other foxes. Weight averages for all foxes were 3.85 kg, with males having averages of 4.06 kg, and females 3.62 kg. Therefore this one very large fox was removed from all statistical analyses.

Table 2.1 summarizes the means and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ values and C:N ratios for all tissues from our sample of free-ranging red foxes from Bethel Alaska. T-tests showed no difference between male and female mean $\delta^{13}C$, $\delta^{15}N$ and C:N values for any tissues. Table 2.2 summarizes the means and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ values and C:N ratios for all tissues of males and females red foxes separately by sex, and reports p-values for the t-tests.

Paired *t*-tests did indicate significant differences in means of $\delta^{13}C$ and $\delta^{15}N$ values between certain pairs of tissues. Tables 2.3 (carbon) and 2.4 (nitrogen) report mean differences and p-values for all tissues. For carbon pairwise mean of differences, hair, bone and muscle were significantly different from all other tissues. Liver was significantly different from all other tissues except kidney cortex and medulla. Kidney cortex and medulla were not significantly different.

For nitrogen pairwise mean of differences, hair was significantly different from bone and muscle, but not from liver, kidney cortex and medulla. Bone was significantly different from all tissues. Liver was significantly different all tissues except hair, kidney cortex and medulla. Kidney cortex and medulla were significantly different from all tissues except for hair and liver.

Pearson's product-moment correlation was used to estimate correlations in isotope measurements between pairs of tissues. Correlations are reported in Table 2.5 (carbon) and Table 2.6 (nitrogen) along with p-values for testing correlation significance. All p-values were significantly different from zero. Hair was highly correlated with all tissues except bone. Specifically, high correlations were observed for both $\delta^{13}C$ and $\delta^{15}N$ stable isotope measurements between hair and muscle, liver, kidney cortex and medulla; between muscle and liver, kidney cortex and medulla; between liver and kidney cortex and medulla; and between kidney cortex and kidney medulla. Bone showed a medium correlation with all tissues for both $\delta^{13}C$ and $\delta^{15}N$ stable isotope measurements. Consequently bone is less likely to be useful in predicting stable isotope ratios in other tissues of the red fox.

2.4.2 *Relationship to mercury biomagnification*

Figure 2.2 was developed from the THg study of these same red foxes reported in Dainowski et al. (2015) along with the stable isotope data from this paper. We compared the $\delta^{15}N$ values for the Bethel, Alaska red foxes to the THg in their hair (Dainowski et al., 2015). A correlation between $\delta^{15}N$ values and THg concentrations of hair was observed. As $\delta^{15}N$ increases so does THg. Figure 2.2 shows a pattern of hair $\delta^{15}N$ having a value between 5.00 and 7.00‰, and THg concentration between 1.00 and 3.00 ppm.

2.5 Discussion

The potential food sources of the Western Alaska red fox diet include terrestrial, aquatic, and occasional anthropogenic derived foods. C_3 and C_4 plants (Farquhar et al., 1989; Smith and Epstein, 1971; Vogel and

Van Der Merwe, 1977) play an important part of an animal's diet as the $\delta^{13}C$ ratios differ in the photosynthetic pathway. The ratio of $^{13}C/^{12}C$ isotopes in C_3 plants range from approximately -24.00 to -34.00‰ and C_4 plants from approximately -6.00 to -19.00‰ (O'Leary, 1988). Because the isotopes of these two kinds of plants move up through the food chain, it is possible to suggest the red fox's principal diet is one of terrestrial animals feeding on C_3 plants. This is reasonable for Alaska as the native vegetation is exclusively C_3, such as rice, shrubs and grasses (O'Leary, 1988; Sage et al., 1999). Thus, the herbivores that the red fox is preying on would be eating C_3 plants as indicated by the red fox hair mean value $\delta^{13}C$ of -24.89‰ (Table 2.1). The carbon stable isotope average for our red fox population is $\delta^{13}C$ (-22.56 to -25.69‰).

On the other hand, a farmed red fox diet is isotopically different (Peterson and Fry, 1987). Therefore, stable isotope ratios can distinguish the Western Alaska ecosystem wild foods, such as brown lemmings, from processed foods, such as commercial feed to farmed foxes. Roth and Hobson (2000) report the pellet diet of their farmed foxes contained up to fifty percent carbohydrate, resulting in an average $\delta^{13}C$ isotope fractionation value lower than the Alaskan red fox. C_4 plants ($\delta^{13}C$ isotope values ranging between -10.00 and -16.00‰), such as corn in the feed for domestic animals (Jahren et al., 2006), are part of the commercial North American diet (Jahren and Kraft, 2008). A practical forensic use of $\delta^{13}C$ data would be confirming the identity of the source of commercial feeds (Savory et al., 2014).

Nitrogen stable isotope ratios are also different depending on the trophic levels (Hobson and Welch, 1992), and between terrestrial and marine sources (Fry, 2006; Kelly, 2000; Schoeninger and DeNiro, 1984). There is a tendency to have an increase of 2.00‰ to 4.00‰ at each trophic level, however, this depends on the tissue being examined (Adams et al., 2000; Handley et al., 1999; Szpak et al., 2013). For example, bone collagen, because of its isotopic composition being reflective of a diet over a long period of time, has an $\delta^{15}N$ enrichment value (between predator and prey) around 2.00‰ (Bocherens and Drucker, 2003). Other factors that can influence the nitrogen isotopic composition are the environment and physiological factors of animals (e.g. hair growth and muscle fitness) being studied (Adams et al., 2000; Fry, 2006). For example, in wetter and cooler ecosystems plants would retain ^{15}N and thus have a lower ratio (Fry, 2006; Szpak et al., 2013).

Hair and muscle $\delta^{15}N$ can be used to indicate the trophic level where the red fox is feeding. The red fox in this Western Alaska ecosystem appears to feed mainly on lemmings (which feed on C_3 plants) and voles and is in agreement with other reports (Savory, 2013). We compared our quadricep muscle findings with that of Roth and Hobson (2000) to investigate the difference between free-ranging red foxes and farmed red foxes diet using $\delta^{13}C$ and $\delta^{15}N$ stable isotope measurements. Our stable isotope means for free-ranging Western Alaska red fox diets vary from these commercial fur farmed foxes from Ontario Canada as seen in Table 2.7 (Roth and Hobson, 2000). Our isotope data indicates that the Alaska red foxes have a major diet of C_3 carrying herbivores, such as microtines; whereas, the farmed foxes were feeding on a commercial pellet food diet, higher in carbohydrates. Looking at the $\delta^{15}N$ values, the Alaskan foxes are eating at a lower trophic level which could indicate a greater content of marine protein than is usually found in farmed fox commercial food pellets.

The bone model used in the Prudhoe Bay study indicated the red foxes were eating a varied diet of lemmings with anthropogenic food sources mixed in. Therfore, Prudhoe Bay, Alaska red foxes may be feeding at a lower trophic level related to proximity to a human industrial settlement. The bone model used in the Bethel, Alaska study showed that the red foxes were eating a diet mainly composed of lemmings; therefore, the Bethel, Alaska red foxes were eating at a higher trophic level.

The isotope values of the red fox's diet items versus the THg concentrations found in their hair tissue implies that the THg may be coming through the mixed lemming/aquatic/avian pathway (likely fish or other fish eating birds) for those foxes with the higher mercury contents. Because our free-ranging red foxes should retain a summer signal in their hair, a summer seasonal effect of THg biomagnifications may explain the variation in the stable isotope values. In addition, the hair THg tends to have a wider variation suggesting the diet of the Western Alaska free-ranging red fox populations vary in what they are eating as a major component of their diet.

2.6 Conclusion

This is the first report on red fox stable isotope ratios from Western Alaska. The stable isotope means of tissues are slightly, but significantly different from each of the other tissues. The stable isotope signals from hair can correlate with the stable isotope signal of other tissues. The Western Alaska red fox differs in trophic position with the farmed red fox and other free-ranging European red foxes based on differences in their diet patterns. While the $\delta^{13}C$ and $\delta^{15}N$ stable isotope mean value only allows conclusions regarding that tissue, the presence of strong correlations between hair and muscle with the other tissues allows conclusions about the diet and trophic level of the red fox population in Western Alaska. Stable isotope data may explain the mercury levels, which could be influenced by changes in seasonal diet of the red fox. Identifying the trophic position of red fox populations allows for ecological risk assessment of contaminants such as mercury as climate change impacts the physical and biological composition of ecosystems, and/or the migration or interaction of terrestrial species.

2.7 References

Adams, T.S., Sterner, R.W., 2000. The effect of dietary nitrogen content on trophic level ^{15}N enrichment. ASLO 45 (3), 601-607.

Andale, 2015. Pearson Correlation: Definition and Easy Steps for Use. (Retrieved 30 July 2016 from http://www.statisticshowto.com/what-is-the-pearson-correlation-coefficient/).

Armstrong, R.H., 1994. Alaska Blackfish. Wildlife notebook series. Alaska Department of Fish and Game, Anchorage, AK.

Bee, J.W., Hall, E.R., 1956. Mammals of northern Alaska on the Arctic Slope. University of Kansas Museum of Natural History Publisher, Lawrence, KS.

Ben-David, M., Flaherty, E.A., 2012. Stable isotopes in mammalian research: a beginner's guide. J. Mammal. 93, 312-328.

Ben-David, M., Newsome, S.D., Whiteman, J.P., 2012. Lipid and amino acid composition influence incorporation and discrimination of ^{13}C and ^{15}N in mink. J. Mammal, 93 (2), 399-412.

Bergeron, C.M., Hopkins, W.A., Bodinof, C.M., Budischak, S.A., Wada, H., Unrine, J.M., 2011. Counterbalancing effects of maternal mercury exposure during different stages of early ontogeny in American toads. Sci. Total Environ. 409, 4746-4752.

Bocherens, H., Drucker, D., 2003. Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: Case studies from recent and ancient terrestrial ecosystems. Int. J. Osteoarchaeol. 13, 46-53.

Bond, A.L., Diamond, A.W., 2010. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol. Appl. 21, 1017-1023.

Brower, C.D., 1942. Fifty years below zero. Dodd, Mead, and Company, New York, NY.

Carroll, S.S., Horstmann-Dehn, L., Norcross, B.L., 2013. Diet history of ice seals using stable isotope ratios in claw growth bands. Can. J. Zool. 91 (4), 191-202.

Chesemore, D.L., 1967. Ecology of the arctic fox in northern and western Alaska. (MS Thesis), University of Alaska Fairbanks, Fairbanks, AK.

Chirkova, A., 1968. The relationship between arctic fox and red fox in the far north. Problems in the North 11, 129-131.

Dainowski, B.H., Duffy, L.K., McIntyre, J., Jones, P., 2015. Hair and bone as predictors of tissular mercury concentration in the Western Alaska Red Fox, *Vulpes vulpes.* Sci. Total Environ. 518-519, 526-533, doi:10.1016/j.scitotenv.2015.03.013

Dalerum, F., Angerbjorn, A., 2005. Resolving temporal variation in vertebrate diets using natural occurring stable isotopes. Oecologia 144, 647-658.

Dehn, L.A., Follmann, E.H., Thomas, D.L., Sheffield, G.G., Rosa, C., Duffy, L.K., O'Hara, T.M., 2006. Trophic relationships in an Arctic food web and implications for trace metal transfer. Sci. Total Environ. 362, 103-123.

Dell'Arte, G.L., Laaksonen, T., Norrdahl, K., Korpimäki, E., 2007. Variation in the diet composition of a generalist predator, the red fox, in relation to season and density of main prey. Acta Oecol. 31, 276-281.

DeNiro, M.J., Epstein, S., 1977. Mechanisms of carbon isotope fractionation associated with lipid synthesis. Science 197, 261-263.

DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Ac. 45, 341-351.

Fagan, K.A., Koops, M.A., Arts, M.T., Power, M., 2011. Assessing the utility of C:N ratios for predicting lipid content in fishes. Can. J. Fish. Aquat. Sci. 68 (2), 374-385.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 503-537.

France, R.L., 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar. Ecol. Prog. Ser. 124, 307-312.

Fry, B., 2006. Stable Isotope Ecology. Springer: New York, NY.

Gotthardt, T.A., McClory, J.G., 2006. State Conservation Status, Element Ecology & Life History. Alaska Natural Heritage Program, Environment and Natural Resources Institute, University of Alaska Anchorage, Alaska.

Handley, L.L., Austin, A.T., Stewart, G.R., Robinson, D., Scrimgeour, C.M., Raven, J.A., Heaton, T.H.E., Schmidt, S., 1999. The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects measures of water availability. Aust. J. Plant Physiol. 26, 185-199.

Harris, S., 1981. The food of suburban foxes (*Vulpes vulpes*) with special reference to London. Mammal Rev. 11, 151-168.

Hersteinsson, P., Macdonald, D.W., 1992. Interspecific competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus.* Oikos 64 (3), 505.

Hobson, K.A., Fisk, A.T., Karnovsky, N., Holst, M., Gagnon, J.M., Fortier, M., 2002. A stable isotope ($\delta^{13}C$, $\delta^{15}N$) model for the North Water foodweb: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep Sea Research Part II: Topical studies in Oceanography 49 (22-23), 5131-5150.

Hobson, K.A., Quirk, T.W., 2014. Effect of age and ration on diet-tissue isotopic ($\delta^{13}C$, $\delta^{15}N$) discrimination in striped skunks (*Mephitis mephitis*). Isot. Environ. Healt. S. 50 (3), 300-306.

Hobson, K.A., Welch, H.E., 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar. Ecol. Prog. Ser. 84, 9-18.

Jahren, A.H., Kraft, R.A., 2008. Carbon and nitrogen stable isotopes in fast food: signatures of corn and confinement. Proc. Nat. Acad. Sci. 105, 17855-17860.

Jahren, A.H., Saudek, C., Yeung, E.H., Kao, W.H.L., Kraft, R.A., Caballero, B., 2006. An isotopic method for quantifying sweeteners derived from corn and sugar cane. Am. J. Clin. Nutr. 84, 1380-1384.

Kalisinska, E., Lisowski, P., Salicki, W., Kucharska, T., Kavetska, K., 2009. Mercury in wild terrestrial carnivorous mammals from north-western Poland and unusual fish diet of red fox. Acta Theriol. 54 (4), 345-356.

Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78, 1-27.

Kielland, K., 2001. Stable isotope signatures of moose in relation to seasonal forage composition: a hypothesis. Alces 37, 329-337.

Kiener, T.V., Zaitsev, V.A., 2010. Range structure in the red fox (*Vulpes vulpes* L.) in the forest zone of Eastern Europe. Contemporary Problems of Ecology 3 (1), 119-126.

Larivière, S., Pasitschniak-Arts, M., 1996. *Vulpes vulpes*. Mammalian Species 537, 1-11.

Lecomte, N., Ahlstrøm, Ø., Ehrich, D., Fuglei, E., Ims, R.A., Yoccoz, N.G., 2011. Intrapopulation variability shaping isotope discrimination and turnover: experimental evidence in arctic foxes. PLoS ONE 6(6): e21357. doi:10.1371/journal.pone.0021357.

Lehner, N., 2012. Arctic fox winter movement and diet in relation to industrial development on Alaska's north slope. (MS Thesis), University of Alaska Fairbanks, Fairbanks, AK.

Lindström, E.R., 1983. Condition and growth of red foxes *Vulpes vulpes* in relation to food supply. J. Zool. (Lond.) 199, 117-122.

Lovejoy, T.E., Hannah, L., 2006. Climate change and biodiversity. Yale University Press, USA.

Matheus, P.E., 1997. Paleoecology and ecomorphology of the giant short-faced bear in Eastern Beringia. PhD Dissertation, University of Alaska, Fairbanks.

Maurel, D., Coutant, C., Boissin-Agasse, L., Boissin, J., 1986. Seasonal moulting patterns in three fur bearing mammals: the European badger (*Meles meles* L.), the red fox (*Vulpes vulpes* L.), and the mink (*Mustela vison*). A morphological and histological study. Can. J. Zool. 64 (8), 1757-1764.

McCutchan, J.H., Lewis Jr, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. OIKOS 102, 378-390.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between Delta ^{15}N and animal age. Geochim. Cosmochim. Ac. 48, 1135-1140.

Murray, M.S., McRoy, C.P., Duffy, L.K., Hirons, A.C., Schaaf, J.M., Trocine, R.P., Trefry, J., 2015. Biogeochemical analysis of ancient Pacific cod bone suggest Hg bioaccumulation was linked to paleo sea level rise and climate change. Front. Environ. Sci. 3 (8), 1-8.

Newsome, S.D., Wolf, N., Peters, J., Fogel, M.L., 2014. Amino Acid $\delta^{13}C$ Analysis Shows Flexibility in the Routing of Dietary Protein and Lipids to the Tissue of an Omnivore. ICB 54 (5), 890-902.

O'Leary, M.H., 1988. Carbon Isotopes in Photosynthesis. BioScience 38 (5), 328-336.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18, 293-320.

Phillips, D.L., Eldridge, P.M., 2006. Estimating the timing of diet shifts using stable isotopes. Oecologia 147 (2), 195–203. doi:10.1007/s00442-005-0292-0.

Post, D.M., 2002. Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. Ecology 83 (3), 703-718.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179-189.

R Development Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria 3-900051-07-0 (Retrieved on 7 Oct 2015 from http:// www.R-project.org).

Rau, G.H., Ainley, D.G., Bengston, J.L., Torres, J.J., Hopkins, T.L., 1992. '5N/'4N and '3C/'2C in Weddell Sea birds, seals, and fish: implications for diet and trophic structure. Mar. Ecol. Prog. Ser. 84, 1-8.

Reeves, M.K., Trust, K.A., 2008. Contaminants as Contributing Factors to Wood Frog Abnormalities on the Kenai National Wildlife Refuge, Alaska. Final Report. U.S. Fish and Wildlife Service Technical Report. AFWFO TR#2008-01. pp. 257.

Robbins, C.T., Felicetti, L.A., Sponheimer, M., 2005. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144, 534-540.

Roth, J.D., Hobson, K.A., 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. Can. J. Zool. 78 (5), 848-852.

Roth, J.D., Marshall, J.D., Murray, D.L., Nickerson, D.M., Steury, T.D., 2007 Geographical Gradients in Diet Affect Population Dynamics of Canada Lynx. Ecology 88 (11), 2736-2743.

Rounick, J.S., Winterbourn, M.J., 1986. Stable carbon isotopes and carbon flow in Ecosystems. Bioscience 36 (3), 171-177.

Ruan, D., Chen, G., Kerre, E., 2005. Intelligent Data Mining: Techniques & Applications. In: Wets G (ed.) Studies in Computational Intelligence. Vol 5. Springer. pp. 318.

Sage, R.F., Wedin, D.A., Li, M., 1999. The biogeography of C4 photosynthesis: patterns and controlling factors. In: Sage RF and Monson RK (eds.) C4 plant biology. Academic Press, San Diego. pp, 596.

Sargeant, A.B., 1978. Red Fox Prey Demands and Implications to Prairie Duck Production. J. Wildl. Manage. 42 (3), 520-527.

Savory, G., 2013. Foxes and Food Subsidies: Anthropogenic food use by red and arctic foxes, and effects on arctic fox survival, on the arctic coastal plain of Alaska. (MS Thesis), University of Alaska Fairbanks, Fairbanks, AK.

Savory, G.A., Hunter, C.M., Wooller, M.J., O'Brien, D.M., 2014. Anthropogenic food use and diet overlap between red foxes (*Vulpes vulpes*) and arctic foxes (*Vulpes lagopus*) in Prudhoe Bay, Alaska. Can. J. Zool. 92, 657-663.

Schoeninger, M.J., DeNiro, M.J., 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim.Cosmochim. Ac. 48, 625-639.

Smith, B.N., Epstein, S., 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiol. 47, 380-384.

Sweeting, C.J., Jennings, S., Polunin, M.V.C., 2005. Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivore. Funct. Ecol. 19, 777-784.

Szpak, P., White, C.D., Longstaffe, F.J., Millaire, J.F., Vásquez Sánchez, V.F., 2013. Carbon and Nitrogen Isotopic Survey of Northern Peruvian Plants. Baselines for Paleodietary and Paleoecological Studies. PLOS ONE 8, e53763.

Tesky, J.L., 1995, *Vulpes vulpes*. In: Fire Effects Information System, [online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). (Retrieved on 10 Nov 2015 from http://www.fs.fed.us/database/feis/).

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., Slade, N.A., 1983. Fractionation and turnover for $\delta^{13}C$ analysis of diet in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Ecology 57 (1), 32-37.

Vogel, J.C., Van Der Merwe, N.J., 1977. Isotopic evidence for early maize cultivation in New York State. Am. Antiquity 42, 238-242.

von Schantz, T., 1980. Prey consumption of a red fox population in Southern Sweden. Biogeographica 18, 53.

2.8 Figures

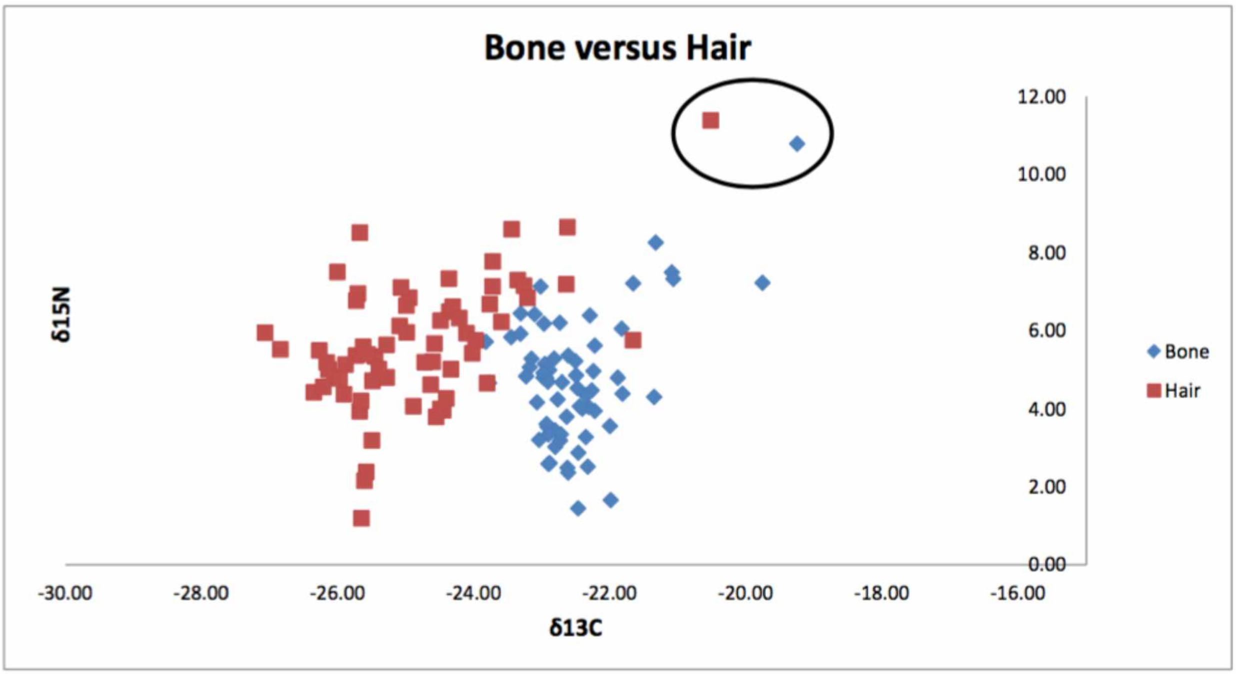

Figure 2.1: $\delta^{13}C$ versus $\delta^{15}N$ levels for hair and bone in western Alaska wild red foxes. One male fox outlier is circled.

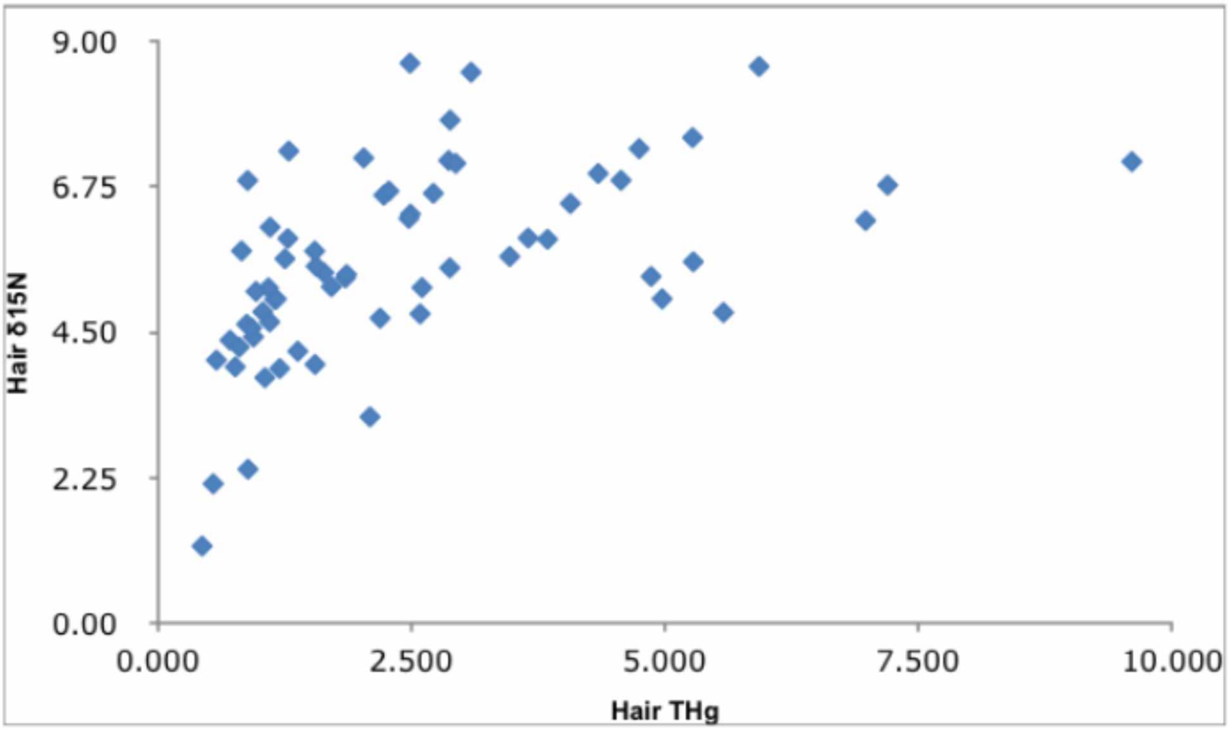

Figure 2.2: $\delta^{15}N$ versus THg of hair in free-ranging red foxes from western Alaska. THg concentrations are reported in ppm (mg/kg) and $\delta^{15}N$ values are reported in ‰. $R^2 = 0.42$. Mean(std dev) of hair 5.57(1.48), and THg 2.58(1.96). Stable isotope values for hair correlate with THg levels in hair.

2.9 Tables

Table 2.1: Mean and standard deviation for $\delta^{13}C$ and $\delta^{15}N$ and C:N ratios for all red foxes (n=65).

	Hair	Bone	Liver	Muscle	KidneyC	KidneyM
δ^{13}Carbon						
Mean (sd)	-24.89 (1.08)	-22.56 (0.67)	-25.37 (1.34)	-25.69 (1.14)	-25.35 (1.21)	-25.31 (1.21)
δ^{15}Nitrogen						
Mean (sd)	5.57 (1.48)	4.64 (1.47)	5.72 (1.64)	5.20 (1.53)	5.63 (1.66)	5.85 (1.53)
C:N Ratio						
Mean (sd)	2.62 (0.05)	2.51 (0.04)	4.01 (0.61)	3.38 (0.31)	4.02 (0.22)	3.83 (0.30)

Table 2.2: Mean, standard deviation and p-values for $\delta^{13}C$ and $\delta^{15}N$ and C:N ratios for male (n=34) and female (n=31) red foxes.

	Hair	Bone	Liver	Muscle	KidneyC	KidneyM
δ^{13}Carbon						
Male Mean (sd)	-24.82 (0.98)	-22.61 (0.50)	-25.43 (1.06)	-25.72 (0.94)	-25.52 (0.87)	-25.43 (0.86)
Female Mean (sd)	-24.97 (1.20)	-22.50 (0.83)	-25.31 (1.61)	-25.65 (1.35)	-25.16 (1.50)	-25.18 (1.50)
P-value	0.61	0.56	0.72	0.83	0.26	0.42
δ^{15}Nitrogen						
Male Mean (sd)	5.60 (1.42)	4.57 (1.25)	5.80 (1.68)	3.34 (1.48)	5.67 (1.68)	5.86 (1.51)
Female Mean (sd)	5.53 (1.57)	4.74 (1.70)	5.66 (1.61)	5.18 (1.61)	5.58 (1.66)	5.82 (1.58)
P-value	0.85	0.69	0.91	0.94	0.81	0.92
C:N Ratio						
Male Mean (sd)	2.61 (0.06)	2.51 (0.04)	3.93 (0.40)	3.36 (0.30)	4.07 (0.24)	3.91 (0.29)
Female Mean (sd)	2.63 (0.05)	2.51 (0.03)	4.10 (0.79)	3.40 (0.31)	3.97 (0.20)	3.94 (0.32)
P-value	0.16	0.56	0.62	0.64	0.06	0.71

Table 2.3: Carbon stable isotope paired t-test among all tissues. Mean of differences (top number) / p-values (bottom number).

	Hair	Bone	Muscle	Liver	KidneyC	KidneyM
Hair	----	-2.33	0.79	0.483	0.46	0.42
		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Bone		----	3.12	2.81	2.79	2.75
			<0.0001	<0.0001	<0.0001	<0.0001
Muscle			----	-0.311	-0.33	-0.37
				0.001	<0.0001	<0.0001
Liver				----	-0.02	-0.05
					0.791	0.466
KidneyC					----	-0.03
						0.404
KidneyM						----

Table 2.4: Nitrogen stable isotope paired t-test among all tissues. Mean of differences (top number / p-values (bottom number).

	Hair	Bone	Muscle	Liver	KidneyC	KidneyM
Hair	----	0.92	0.37	-0.15	-0.05	-0.27
		<0.0001	<0.0001	0.328	0.696	0.044
	Bone	----	-0.55	-1.08	-0.98	-1.20
			0.002	<0.0001	<0.0001	<0.0001
		Muscle	----	-0.52	-0.42	-0.64
				<0.0001	<0.0001	<0.0001
			Liver	----	0.09	-0.12
					0.002	0.010
				KidneyC	----	-0.21
						<0.0001
					KidneyM	----

Table 2.5: Carbon stable isotope correlations among all tissues. Pearson's product-moment correlation (top number) / p-values (bottom number).

	Hair	Bone	Muscle	Liver	KidneyC	KidneyM
Hair	----	0.423	0.819	0.673	0.709	0.692
		0.0004	<0.0001	<0.0001	<0.0001	<0.0001
Bone		----	0.308	0.328	0.318	0.346
			0.013	0.007	0.010	0.005
Muscle			----	0.838	0.913	0.904
				<0.0001	<0.0001	<0.0001
Liver				----	0.891	0.875
					<0.0001	<0.0001
KidneyC					----	0.952
						<0.0001
KidneyM						----

Table 2.6: Nitrogen stable isotope correlation among all tissues. Pearson's product-moment correlation (top number) / p-values (bottom number).

	Hair	Bone	Muscle	Liver	KidneyC	KidneyM
Hair	----	0.613	0.851	0.680	0.719	0.744
		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Bone	----	0.550	0.405	0.433	0.480
			<0.0001	<0.0001	<0.0001	<0.0001
		Muscle	----	0.928	0.939	0.945
				<0.0001	<0.0001	<0.0001
			Liver	----	0.988	0.975
					<0.0001	<0.0001
				KidneyC	----	0.985
						<0.0001
					KidneyM	----

Table 2.7: Summary of actual stable isotope mean values (std dev) for tissues of Alaskan free-ranging red foxes and Southern Ontario, commercially fed, farmed red foxes (as reported by Roth and Hobson, 2000). All values are reported in parts per mil (‰).

	$\delta^{13}C$ Alaska free-ranging foxes	$\delta^{13}C$ Ontario commercial fed farmed foxes	$\delta^{15}N$ Alaska free-ranging foxes	$\delta^{15}N$ Ontario commercial fed farmed foxes
Bone	-22.56 (0.67)	----	4.64 (1.47)	----
Kidney Medulla (KidneyM)	-25.31 (1.21)	----	5.85 (1.53)	----
Kidney Cortex (KidneyC)	-25.35 (1.21)	----	5.63 (1.66)	----
Liver	-25.37 (1.34)	-18.30	5.72 (1.64)	8.20
Muscle	-25.69 (1.14)	-17.60	5.20 (1.53)	8.20
Hair (fur)	-24.89 (1.08)	-16.10	5.57 (1.48)	8.10

Chapter 3 A Forensic Evaluation: The use of mercury and stable isotope analysis of museum bone samples to monitor if environmental changes are affecting the eating patterns of red and Arctic foxes[1]

3.1 Abstract

This research takes a forensic approach at investigating certain health and dietary indicators in museum preserved bone of red (*Vulpes vulpes*) and Arctic foxes (*Vulpes lagopus*) from the Yukon Territory in Canada. This study attempts to 1) measure the mercury (THg) concentration levels, 2) estimate a diet using carbon stable isotopes ($\delta^{13}C$) and 3) establish a trophic level using nitrogen stable isotopes ($\delta^{15}N$), from bones of these sentinel species. This study examines two Arctic foxes and three red foxes or unknown age and origin. Yukon Territory Arctic foxes THg concentrations were 0.017 and 0.025 mg/kg. The red foxes THg concentrations were 0.010, 0.036 and 0.073 mg/kg. The $\delta^{13}C$ levels were -21.13 and -21.36‰ for Arctic foxes and -20.05, -20.08, and -23.12‰ for red foxes. Their $\delta^{15}N$ levels were 5.59 and 7.22‰ for the Arctic foxes and 6.10, 6.57 and 6.66‰ for red foxes. These Arctic and red Yukon Territory foxes indicate a trophic level similar to Arctic terrestrial omnivores.

---

[1] Dainowski BH, Duffy LK (2016). A Forensic Evaluation: The use of mercury and stable isotope analysis of museum bone samples to monitor if environmental changes are affecting the eating patterns of red and Arctic foxes. Submitted for book chapter publication in Contamination in the Soil Environment. Dr Ashutosh Gautam and Dr Chakresh Pathak (Eds). India Glycols Limited.

3.2 Introduction

Forensic science uses observations and analytical techniques to measure data from wildlife remains. The commonly used techniques include toxicology and stable isotope analysis of animal bone (Meier-Augenstein 2008, NRC 2009). The impact of mercury (THg) to the environment is increasing. Its ability to build up in organisms and food webs has a significant influence on the health of animals. Understanding THg contamination in the environment can help prevent ecological effects of biological diversity causing possible loss of ecosystems and local animal populations in the Arctic region. Stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition of animal tissue has also been successful in establishing a relationship between diet, geographic location and trophic levels in archaeological and palaeodietary studies (Meier-Augenstein 2008, NRC 2009). This information yields valuable life history and geographic origins of animals.

The slower decay rates of bone makes it more readily available than soft tissues for analysis (Doyle 1979, Lehner 2012, Nielsen-Marsh and Hedges 1999). An extensive baseline study of THg concentrations (Dainowski et al. 2015), and stable isotopes (unpublished data) in modern red fox bones from Alaska and throughout Europe has been established. This modern collection of animal bones has been useful in evaluating current toxicant levels, diets, and trophic levels in the far North. Since bones are available in museum collections around the North, they can be used to understand the past diets of wildlife. These established baselines will help to monitor future toxicant and dietary changes due to climate changes facing our Northern environment.

The study presented here starts a database from a museum bone collection for the red and Arctic fox relating to climate and industrialization changes in the far North. It is a collaborative study, with graduate student Garrett Savory, who has reported on Arctic foxes (Savory 2013, Savory et al. 2014) to examine the application of wildlife forensics from THg and $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis by using museum samples. We examined archived museum samples from The Yukon Fossil Collection. These museum bones were supplied by Dr. Grant Zazula, a Yukon Palaeontologist with the Heritage Resources Unit, Cultural Services Branch, Department of Tourism & Culture, Government of Yukon. The age of the bones are unknown, and could range from modern to historic. Thus, we will use the term 'fossil' when referring to these museum bones from the Yukon Territory in Canada per Dr. Zazula's estimations. We report the THg accumulation and $\delta^{13}C$ and $\delta^{15}N$ levels to try to understand the fox's past feeding ecology and trophic levels in their changing environment. We report the THg and stable isotope data and identify the trophic levels of two Arctic and three red fox bone samples. The red foxes from the Yukon Fossil Collection will be compared to that of the modern red fox bones from Bethel Alaska in a previous study on THg concentrations (Dainowski et al. 2015) and stable isotopes (unpublished data).

3.2.1 *Terrestrial mammal bones used in mercury studies*

An important resource for exploring diets of past wildlife populations is archival bones from museums. Museum bones can establish baselines for different areas of the world, and be used to predict future dietary changes of wildlife populations. This research will try to determine if contaminants, such as THg, are possibly increasing due to climate changes in the Arctic, and to establish a historical baseline for contaminants based upon assumption of the age of the bones. It is important to establish such a baseline as the release of THg into the environment, with potential methylation, the foxes tissues will likely accumulate THg concentrations, causing damage to the animals organ systems (Dainowski et al. 2015). One more report exists on THg in wild northern red fox omnivore bones in addition to Dainowski et al. (2015) (Lanocha et al. 2012). Where Lanocha and colleagues (2012) did a study on the THg concentrations in bones and the effects on the osseous–articular system.

3.2.2 *Terrestrial mammal bones used in stable isotope studies*

Over the last several decades, stable isotopes have been developed as tools for studying animal migration patterns, as well as movements of elements through the biosphere. Stable isotopes of carbon and nitrogen levels are used to reconstruct the dietary life history of individual animals (Hobson 1999, 2005, Lehner 2012) to inform wildlife management policy. Stable isotope tracers in environmental research incorporate stable isotope signals into landscape maps (Crawford et al. 2008, Gannes et al. 1997). Also stable isotopes have been useful in studying the feeding habits of species, and are valuable chemical descriptors of niche space, food web organization, nutritional shifts, and community ecology (Dehn et al. 2006, DeNiro and Epstein 1978, Fry 2006, Gannes et al. 1997, Robbins et al. 2005). Bone collagen has been used in several diet studies to determine if an animal is eating from an exclusive marine environment or not (Bell et al. 2001, Jim et al. 2004, Schoeninger and DeNiro 1984, Stevens et al. 2006). One paleoecology study used bone collagen to predict trophic levels in red deer (Stevens et al. 2006). Another study used ancient and modern bone collagen from a lynx and wolf, from Bialowieza primeval forest in Poland, to predict trophic levels (Bocherens and Drucker 2003). Growth studies, faunal analysis, and paleopathology are just a few nutritional assessment studies that can provide information of past diets by stable isotope analysis of bone collagen (Ben-David and Flaherty 2012, Klepinger 1984).

3.2.3 *Diagenesis of bone*

Diagenesis is the breakdown of bone and its interaction with the local physical, chemical and biological environment over time (Collins et al. 2002). These processes modify the bone’s original structural and chemical properties and can either preserve or destroy the bone. Physical factors such as soil and climate, chemical factors such as deterioration of the organic and mineral phases, and biological factors such as alterations that take place on the bone itself in a burial context, all are part of the diagenesis process (Hedges 2002). It has been reported that mercury invades the porosity portion of bone during diagenesis (Nielsen-Marsh and Hedges 1999).

Bones are not in equilibrium with the soil solution of that particular environment and therefore undergo a chemical deterioration (Collins et al. 2002, Dietz et al. 2009). Bone exposed to moist environmental conditions, a key agent of change in diagenesis, is altered in the proportions of the inorganic components (e.g. calcium, hydroxyapatite, magnesium) and the organic component (e.g. collagen). Various types of soil components absorb onto the bone surface and cause the components of bone to leach out (Hedges 2002, Vass 2001). Studies have described techniques for the preservation of bone, classification of soil environment, and detection of the factors in the environment which affect the preservation of bone (Dietz et al. 2009, Jans et al. 2002).

In one forensic study in Japan, mercury was detected in excavated bones (Yamada et al. 1995). Under dry conditions, $\delta^{13}C$ and $\delta^{15}N$ values change less than 0.4‰ during the critical first 195 hours and in the presence of excess water, $\delta^{13}C$ and $\delta^{15}N$ values change up to 1.0‰ and 0.5‰, respectively (McNulty et al. 2002). On the other hand, Schoeninger et al. (1989) argue that a low yield of collagen does affect stable isotope values, while Hare et al. (1991) showed that bone with less than a 5% protein content has a non-collagenous amino acid composition, and therefore is not valid for studies.

3.3 Material and Methods

3.3.1 *Study area*

Figure 3.1 shows the Yukon Territory in Canada. The Arctic and red fox bones that were found in the Yukon Territory in Canada, are part of the Yukon Fossil Collection and are listed in Table 3.1. Herschel Island, Yukon Territory, is a cultural site located in the Beaufort Sea, part of the Arctic Ocean. It is approximately five kilometers from the coast of the Yukon at the most northern region of the territory ("National Trust for Canada" 2016, "VirtualMuseum" 2016). Allgold Creek is a small body of running water, a stream, in the Yukon Territory in Canada ("Traveling Luck" 2016) Quartz Creek is a tributary of the Indian River Drainage (part of Bering Sea drainage basin and a tributary of the Yukon River) in the Yukon Territory in Canada ("Indian River (Yukon)" 2016). Both creeks, Quartz Creek in the Klondike district and Allgold Creek in Dawson City area, have a history of gold mining ("Dulac Mining" 2016, "Mindat.org" 2016). While Herschel Island is a coastal tundra ecosystem, the Yukon River drainage is Boreal Forest.

3.3.2 *Sample preparation and mercury analysis*

Bones were cored under a fume hood while wearing a 3 M™ particulate respirator N95. The bones were cored using a Dremel™ glass diamond drilling bit, 1/4″ (6.350 mm), 663DR, drill speed #23. Each bone was drilled three locations to produce six cores. The periosteum and any trabecular bone were removed using a Dremel™ glass diamond taper point sander 3/32″ (2.381 mm), #7144. Total core mass was determined for each individual for a total wet weight (ww). The cores were stored at -20°C in acid-washed (5% HNO_3) vials prior to degreasing.

Prior to mercury analysis, bone cores were degreased with a series of chloroform treatments in a chemical fume hood (Aerssens et al.1998, Bell et al. 2001, Dwek 2010, Schutkowski and Herrmann 1999, Zwanziger, 1989). Enough chloroform (Chloroform, Reagent ACS, Sciencelab.com) was added to each vial to completely cover bone core samples. Each vial was then loosely capped. Samples were treated for a total of 16 hours, changing chloroform after 8 hours. After chloroform treatments, samples were rinsed several times with ultrapure water. Zwanziger (1989) suggested that freeze drying bone results in decreased THg concentrations, thus degreased samples were air-dried in the fume hood for four days. Bone was homogenized for analysis using a Wig-L-Bug™ (Crescent Dental Company Chicago) with a 9.5 mm stainless steel ball bearing (~10 s).

Approximately 30 mg of homogenized powdered bone was analyzed for THg using a 3-cell low detection DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, Connecticut, USA; USA EPA method 7473). The low detection DMA-80 was calibrated using a 6 point linear calibration curve from 0.25 ng to 6.00 ng; $R^2 = 0.9999$, resulting in a minimum detection limit of 0.008 μg/g. All analytical runs included measurement of blanks, liquid standards (1 μg/g, 0.1 μg/g, 0.01 μg/g) and a reference material, SRM 1486 (Dainowski et al. 2015).

3.3.3 *Sample preparation and stable isotope analysis*

Bones were cored under a fume hood while wearing a 3M™ particulate respirator N95. The bones were cored using a Dremel™ glass diamond drilling bit, ¼” (6.350mm), 663DR, drill speed #23. Each femur was drilled completely through the shaft in three locations to produce six core. Four cores were used in a similar study of total mercury (Dainowski et al. 2015), and the remaining 2 cores were used in this stable isotope study. The periosteum and any traebecular bone were removed using a Dremel™ glass diamond taper point sander, 3/32” (2.381mm), #7144. The cores were stored at -20°C in acid-washed (5% HNO_3) vials prior to degreasing. The degreasing method used is as follows: bone core samples were sonicated in deionized water in order to remove any debris. Next, they were soaked in chloroform for two 8 hour periods to remove any lipids, then finally rinsed with deionized water and air-dried under a fume hood for 3 days.

The bone collagen extraction was a variation of the methods set forth by Matheus (1997). The method is as follows: bone samples were demineralized by soaking in an HC1-H_2O solution until they were translucent. After which they were rinsed with deionized water. In order to separate the collagen from other organic compounds and proteins, the bone samples were gelatinized by placing the collagen samples, with an HC1-H_2O solution (pH between 3.0 and 4.0) in a culture tube. The tube was heated to 70°C, under a stream of nitrogen, to dissolve the collagen. The supernatant was lyophilized to retrieve the collagen after centrifugation.

Isotope ratios are presented as stable isotope abundances relative to the international standard (delta (δ) notation) in parts per thousand (permil (‰):

$$\delta X = ((Rsample/Rstandard)-1) \times 10^3, ‰$$

where, element X = ^{13}C or ^{15}N and R is the molar ratios of heavy to light isotope of the sample and the standard (Fry 2006). These isotope ratios are expressed in delta notation of per mil relative to the Viennna PeeDee Belemnite international standards for carbon, and atmospheric N for nitrogen. These standard values are measured using a laboratory standard (peptone) calibrated against several certified reference materials (Fry 2006).

Between 0.1 and 0.2 mg of bone collagen samples were placed in tin capsules then loaded into an autosampler. Samples were analyzed for stable carbon and nitrogen isotopes at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks. A continuous-flow isotope ratio mass spectrometry with a Costech ECS4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the Conflo III interface (Thermo Fisher Scientific, Waltham, MA, USA) was used for analyzing. The laboratory standard was measured multiple times between samples to evaluate the accuracy and precision of analyses. Accuracy and precision was assessed using multiple peptone standards. Precision was within 0.3‰ and accuracy was within 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

3.3.4 *Statistical analysis*

The Yukon Fossil Collection consists of a small sample size of two red fox bones, and three Arctic fox bones. Statistical methods should not be applied and statistical inference cannot be made on the basis of such a small sample size. The THg concentrations and $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of the Yukon Fossil Collection bones were analyzed. The findings for the THg concentrations and for the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values are presented in both tables and figures. The two Arctic fox bones from the Yukon

Fossil Collection will not be compared to any study. The Arctic foxes will be added to the red fox tables and figures for a visual analysis only.

3.4 Results

The results of the THg concentrations for the Yukon Fossil Collection are shown in Table 3.1. The results of the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values for the Yukon Fossil Collection are also shown in Table 3.1.

3.4.1 *Yukon Fossil Collection THg concentrations and $\delta^{13}C$ and $\delta^{15}N$ stable isotope values*

The THg concentration results for the Yukon Fossil Collection are listed in Table 3.1. The Thg bone samples vary slightly from 0.010 mg/kg to 0.073 mg/kg for the red foxes and 0.017 mg/kg and 0.025 mg/kg for the Arctic foxes. Stable isotope analysis for the Yukon Fossil Collection results are shown in Table 3.1. The stable isotope bone samples also vary slightly from -20.05‰ to -23.12‰ for the red foxes $\delta^{13}C$ values and -21.13‰ to -21.36‰ for the Arctic foxes $\delta^{15}N$ values.

3.4.2 *Yukon Fossil Collection verses Bethel, Alaska for THg and $\delta^{13}C$ and $\delta^{15}N$ stable isotopes*

The THg concentrations in modern red foxes from Bethel, Alaska study (Dainowski et al. 2015) was compared to the Yukon Fossil Collection bones of red foxes from Yukon, Canada. Two red fox bones from the Yukon Fossil Collection, YG 97.3 THg concentration of 0.036 mg/kg and YG 97.4 THg concentration of 0.073 mg/kg, were outside the maximum THg value that was reported for the Bethel, Alaska red foxes at 0.010 mg/kg. The Arctic foxes, from the Yukon Fossil Collection, THg concentrations were 0.017 mg/kg for YG 12.49 and 0.025 mg/kg for YG 155.2.

The $\delta^{13}C$ and $\delta^{15}N$ stable isotope values in modern red foxes from Bethel, Alaska study (Dainowski et al. in Chapter 2) was compared to the Yukon Fossil Collection bones of red foxes from Yukon, Canada. In comparing both red fox collections we see the Yukon red foxes $\delta^{13}C$ values (Table 3.1) are higher than those of the Bethel, Alaska study. The Arctic foxes, from the Yukon Fossil Collection, $\delta^{13}C$ values were -21.13‰ for YG 12.49 and -21.36‰ for YG 155.2. The $\delta^{15}N$ values for the Yukon, Canada red fox samples as seen in Table 3.1, are lower than those of the Bethel, Alaska study. The Arctic foxes, from the Yukon Fossil Collection, $\delta^{15}N$ values were 5.59‰ for YG 12.49 and 7.22‰ for YG 155.2.

3.5 Discussion

The Yukon Territory fox bone samples from the Yukon Territory Fossil Collection are rare and not well documented for this large territory (Figure 3.1). There is no background information about these fox bones housed at the Yukon museum in the Department of Tourism & Culture with the Government of Yukon. Were the bones found along the beach? Just sitting on the ground? Any soil samples associated with these bones? How were they handled before taken to the museum? How were they stored at the museum? How old are the bones? Do different bones, (i.e. femur, tibia, mandible) yield different THg concentrations and/or $\delta^{13}C$ and $\delta^{15}N$ values? Additionally, there is an unequal number in the Yukon Fossil Collection data set (e.g. three red foxes and two Arctic foxes); all five (5) fox bones come from four (4) different areas in the Yukon Territory, Canada, as well as no indication of the sex for these fox bones. These many unanswered questions prevent making statistical analyses and assessments. Therefore, we can only provide a visual comparison in Figures 2 and 3 and speculate with the caution that we have only five samples from four different locations in the Yukon Territory (Table 3.1). We also have made very general assessments about THg concentration levels as well as $\delta^{13}C$ and $\delta^{15}N$ levels for dietary and trophic level estimation from these bones. Overall, Figures 3.2 and 3.3 demonstrate that these five fossil samples fall within the expected values reported for the red foxes in the Bethel region of Alaska (Dainowski et al. 2015).

In comparing both red fox collections we see the Yukon red foxes THg concentrations, as seen in Table 3.1, are ten times higher in mercury than the average reported for the Bethel red foxes of 0.004 mg/kg (Dainowski et al. 2015). Two Yukon Territory red foxes, YG 97.3 and YG 97.4, seem to be eating at a higher trophic level than their counterparts, the Yukon Territory Arctic foxes. A possible explanation is that one red fox in particular, YG 97.4, having 0.073 mg/kg THg concentration, might be obtaining mercury from the environment since it was found in Allgold Creek area. Old mining areas in the North often leave mercury and other metals behind from their activities (Ben-David et al. 2001). The Yukon Territory red fox, YG 97.3, also from Allgold Creek area, and with 0.036 mg/kg THg concentration level, also has higher mercury concentrations from the same mining area. Additionally, these two red foxes could also be eating trash, from the mining area, which could contain fish, such as canned tuna, an aquatic source usually higher in mercury. The Yukon Territory red fox, YG 130.65, as well as both Yukon Territory Arctic foxes, YG 12.49 and YG 155.2, seem to be eating a less THg contaminated diet at Quartz Creek. Even though Quartz Creek is another mining area in the Yukon Territory, this red fox has the lowest THg level (Table 3.1). This lower THg level may be indicative of this particular bone found before the mine was established. In addition, by one red fox having a low THg of .010 mg/kg at Quartz Creek, in contrast to the two red foxes, YG 97.3 and YG 97.4, having higher THg levels at Allgold Creek, we have demonstrated that a forensic approach can provide evidence of THg concentration differences in two different localities, yet two similar gold mining areas, in the Yukon Territory of Canada.

One solution to identify any THg uptake in an animals diet is to collect soil samples. Soil samples would help identify any toxicants that might be affecting plant life; thus, ultimately affecting the health of wildlife that consumes any of these contaminated plants and/or berries. One would also ask, is the increased localized contamination due to industrial activity? Are the wastes from industrialization spreading further out in the environment or contained within a specific area? With bones from various time frames, and in the same general location, as well as surrounding soil to analyze, it can be shown that it is possible to determine if the environment is changing. This would help to answer the following future questions: Are the animals migrating north due to a changing environment? If so, are movement patterns of animals affecting their diet? Does this new diet affect the trophic positions of the red and Arctic fox?

At Quartz Creek, fox YG 130.65, has a higher $\delta^{13}C$ stable isotope value (Table 3.1) and in Figure 3.2, it clearly shows the $\delta^{13}C$ stable isotope of this Quartz Creek red fox is similar in grouping as the Bethel Alaska red foxes, tending toward a terrestrial mammal diet, such as voles. In contrast, Figure 3.2 shows the $\delta^{13}C$ values for both the Yukon Territory Arctic foxes and the two Allgold Creek red foxes, YG 97.3 and YG 97.4, tend toward a salmon migration or aquatic diet.

For $\delta^{15}N$ values, there is a tendency to have an increase of 2.00-4.00‰ at each trophic level, however, this depends on the tissue being examined (Adams and Sterner 2000, Handley et al. 1999, Szpak et al. 2013). Bone collagen, because of its isotopic composition being reflective of a diet over a lifetime, has a $\delta^{15}N$ enrichment value (between predator and prey) as being around 2.00‰ (Bocherens and Drucker 2003). Other factors that can influence the $\delta^{15}N$ composition is the environment and physiological factors of animals (e.g. hair growth and muscle fitness) (Adams and Sterner 2000, Fry 2006). For example, in moister and cooler ecosystems plants would retain $\delta^{15}N$ and thus have a lower $^{14}N/^{15}N$ ratio (Fry 2006, Szpak et al. 2013). The $\delta^{15}N$ indicates that the red foxes from Bethel, Alaska are eating at a lower trophic level which could indicate a herbivore influence (Dainowski et al. 2015). The $\delta^{15}N$ values of the Yukon Territory red and Arctic foxes are in line with a trophic level of Arctic terrestrial omnivores like wolves (McGrew et al. 2013), and may tend toward a slight salmon diet.

The museum sample numbers were very small. However, this provided a good starting point using a forensic method to make predictions of diet and trophic levels during climate changes in the Arctic.

Stable isotope data can explain the mercury levels, which can be influenced by changes in seasonal diet of the red fox. The THg study of the Bethel, Alaska red foxes reported in Dainowski et al. (2015) along with the stable isotope data (unpublished data) has shown that as $\delta^{15}N$ increases so does THg. These isotope values of the red fox's diet items versus the THg concentrations implies that the THg may be coming through the mixed lemming/aquatic/avian pathway (likely fish or other fish eating birds) for those foxes with the higher mercury contents (Tavares et al. 2008).

Identifying the trophic positions of the red and Arctic fox populations allows for risk assessment of contaminants that mercury is not a problem now (based upon Bethel, Alaska red fox study) or possibly in the past (based upon unknown sources of contamination in the Yukon Territory bones). But as climate change impacts the physical and biological composition of ecosystems, and/or the migration or interaction of terrestrial species change, the situation might change suggesting continued monitoring of these species.

3.6 Conclusion

There are only three red foxes and two Arctic foxes in the Yukon Fossil Collection and by no means can this be a definitive analysis of all foxes in the Yukon Territory of Canada. The samples in this study were poorly characterized so only limited interpretation was possible. However, we showed that museum specimens are valuable because 1) metals can be detected, 2) bone collagen from fossil bones is preserved and thus stable isotopes can be detected and 3) comparisons to extant populations can be determined. While limited, this study supports the concept of red foxes as a useful sentinel species distinguishing between climate change in the North and industrial contamination. Forensic approaches can inform managers by providing increases in scale in the understanding of climate impacts on mammalian species.

3.7 References

Adams, T.S. and Sterner, R.W. (2000). The effect of dietary nitrogen content on trophic level ^{15}N enrichment. American Society of Limnology and Oceanography 45 (3): 601-607.

Aerssens,J., Boonen,S., Lowet, G. and Dequeker, J. (1998). Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. Endocrinology 139: 663-670.

Bell,L.S., Cox, G., and Sealy, J. (2001). Determining isotopic life history trajectories using bone density fractionation and stable isotope measurements: a new approach. American Journal Physical Anthropology 116: 66-79.

Ben-David, M. and Flaherty, E. (2012). Stable isotopes in mammalian research: a beginner's guide. Journal of Mammalogy 93 (2): 312-328.

Ben-David,M., Duffy,L.K., Glundell, G.M. and Bowyer, R.T. (2001). Natural exposure of coastal river otters to mercury: Relation to age, diet, and survival. Environmental Toxicology and Chemistry 20 (9): 1986-1992.

Bocherens, H. and Drucker, D. (2003). Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. International Journal of Osteoarchaeology 13 (1−2): 46-53.

Collins,M.J., Nielsen–Marsh,C.M., Hiller,J., Smith,C.I., Roberts,J.P., Prigodich,R.V., Wess,T.J., Csapo,J., Millard, A.R. and Turner–Walker, G., (2002). The survival of organic matter in bone: a review. Archaeometry 44 (3): 383-394.

Crawford,K., McDonald, R.A., and Bearhop, S. (2008). Applications of stable isotope techniques to the ecology of mammals. Mammal Review 38 (1): 87-107.

Dainowski,B.H., Duffy,L.K., McIntyre, J., and Jones, P. (2015). Hair and bone as predictors of tissular mercury concentration in the Western Alaska Red Fox, *Vulpes vulpes.* Science of the Total Environment 518-519: 526-533, doi:10.1016/j.scitotenv.2015.03.013

Dehn,L.A., Follmann,E.H., Thomas,D.L., Sheffield,G.G., Rosa,C., Duffy, L.K. and O'Hara, T.M. (2006). Trophic relationships in an Arctic food web and implications for trace metal transfer. Science of the Total Environment 362: 103-123.

DeNiro, M.J. and Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42 (5): 495-506.

Dietz,R., Outridge, P.M. and Hobson, K.A. (2009). Anthropogenic contributions to mercury levels in present-day Arctic animals — a review. Science of the Total Environment 407: 6120-6131.

Doyle, J.J. (1979). Toxic and essential elements in bone — a review. Journal of Animal Science. 49: 482-49.

Dulac Mining. *'Home'*. (2016). Retrieved May, 23 2016 from http://www.dulacmining.com/

Dwek, J. (2010). The periosteum: what is it, where is it, and what mimics it in its absence? Skeletal Radiology 39: 319-323.

Fry, B. (2006). Stable Isotope Ecology. Springer-Verlag New York.

Gannes,L.Z., O'Brien, D.M. and Martínez del Rio, C. (1997). Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78 (4): 1271-1276.

Handley,L.L., Austin,A.T., Stewart,G.R., Robinson,D., Scrimgeour,C.M., Raven,J.A., Heaton, T.H.E. and Schmidt, S. (1999). The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects measures of water availability. Australian Journal of Plant Physiology 26: 185-199.

Hare,P.E., Fogel,M.L., Stafford,T.W., Mitchell, A.D. and Hoering, T.C. (1991). The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. Journal of Archaeological Science 18 (3): 277-292.

Hedges, R.E.M. (2002). Bone Diagenesis: An Overview of Processes. Archaeometry 44 (3): 319-328.

Hobson, K.A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120 (3): 314-326.

Hobson, K.A. (2005). Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. The Auk 122 (4): 1037-1048.

Hobson,K.A., McLellan, B.N. and Woods, J.G. (2000). Using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to infer trophic relationships among black and grizzly bears in the upper Columbia River basin, British Columbia. Canadian Journal of Zoology, 78 (8): 1332-1339.

Indian River (Yukon). (2016). Retrieved March, 9 2016 from https://en.wikipedia.org/wiki/Indian_River_(Yukon)

Jans,M.M.E., Kars,H., Nielsen–Marsh,C.M., Smith,C.I., Nord,A.G., Arthur, P. and Earl, N. (2002). In situ preservation of archaeological bone: a histological study within a multidisciplinary approach. Archaeometry 44 (3): 343-352.

Jim,S., Ambrose, S.H. and Evershed, R.P. (2004). Stable carbon isotopic evidence for differences in the dietary origin of bone cholesterol, collagen and apatite: implications for their use in palaeodietary reconstruction. Geochimica et Cosmochimica Acta 68 (1): 61-72.

Klepinger, L.L. (1984). Nutritional assessment from bone. Annual Review of Anthropology 13: 75-96.

Lanocha,N., Kalisinska,E., Kosik-Bogacka,D.I., Budis, H., Noga-Deren, K. (2012). Trace metals and micronutrients in bone tissues of the red fox *Vulpes vulpes* (L., 1758). Acta Theriologica 57: 233-244.

Leconte,N., Ahlstrom,O., Ehrich,D., Fuglei,E., Ims, R.A. and Yoccoz, N.G. (2011). Intrapopulation variability shaping isotope discrimination and turnover: experimental evidence in Arctic Foxes. PLoS ONE 6:e21357. dos:10.1371/journal.pone.0021357

Lehner, N. (2012). Arctic Fox Winter Movement and Diet in Relation to Industrial Development on Alaska's North Slope (MS Thesis). University of Alaska Fairbanks, Fairbanks, AK.

Matheus, P.E. (1997). Paleoecology and ecomorphology of the giant short-faced bear in Eastern Beringia. (PhD Dissertation), University of Alaska Fairbanks, Fairbanks, AK.

McGrew,A., Ballweber,L., Moses,S., Stricker,C., Beckmen,K., Salman, M. and O'Hara, T. 2013. Mercury in gray wolves (*Canis lupus*) in Alaska: increased exposure through consumption of marine prey. Journal of the Total Environment 468-469, 609-613.

McNulty,T., Calkins,A., Ostrom,P., Gandhi,H., Gottfried,M., Gage, D. and Martin, L. (2002). Stable isotope values of bone organic matter: Artificial diagenesis experiments and paleoecology of Natural Trap Cave, Wyoming. Palaos 17: 36-49.

Meier-Augenstein, W. and Fraser, I. (2008). Forensic Isotope analysis leads to identification of a mutilated murder victim. Science and Justice 48: 153-159.

Mindat.org. (2016). *'Klondike Area (Klondyke Districk; Klondike District), Dawson Mining Districts, Yukon Canada'.* Retrieved June, 23 2016 from http://www.mindat.org/loc-122872.html

National Trust for Canada. (2016). *'Herschel Island'*. Retrieved April, 11 2016 from https://www.nationaltrustcanada.ca/issues-campaigns/top-ten-endangered/explore-past-listings/territories/herschel-island

Neil, C. and Cornwell, J.C. (1992). Stable carbon, nitrogen, and sulfur isotopes in a prairie marsh food web. Wetlands 12: 217-224.

Nielsen-Marsh, C.M. and Hedges, R.E.M. (1999). Bone porosity and the use of mercury intrusion porosimetry in bone diagenesis studies. Archaeometry 41: 165-174.

NRC (2009). http://www.nrc.gov/reading-rm/doc-collections/nuregs/pubs/2009/

Robbins,C.T., Felicetti, L.A. and Sponheimer, M. (2005). The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144 (4): 534-540.

Roth,J.D., Marshall,J.D., Murray,D.L., Nickerson, D.M. and Steury, T.D. (2007). Geographicgradients in diet affect population dynamics of Canada lynx. Ecology 88 (11): 2736-2743.

Savory, G. (2013). Foxes and Food Subsidies: Anthropogenic food use by red and arctic foxes, and effects on arctic fox survival, on the arctic coastal plain of Alaska. (MS Thesis), University of Alaska Fairbanks, Fairbanks, AK.

Savory,G.A., Hunter,C.M., Wooller, M.J. and O'Brien, D.M. (2014). Anthropogenic food use and diet overlap between red foxes (*Vulpes vulpes*) and arctic foxes (*Vulpes lagopus*) in Prudhoe Bay, Alaska. Canadian Journal of Zoology 92: 657-663.

Schmutz, J.A. and Hobson, K.A. (1998). Geographic, temporal, and age-specific variation in diets of Glaucous Gulls in western Alaska. Condor pp 119-130.

Schoeninger,M.J., Moore,K.M., Murray, M.L. and Kingston, J.D. (1989). Detection of bone preservation in archaeological and fossil samples. Applied Geochemistry 4 (3): 281-292.

Schoeninger, M.J. and DeNiro, M.J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta 48 (4): 625-639.

Schutkowski, H. and Herrmann, B. (1999). Diet, status and decomposition at Weingarten: trace element and isotope analyses on early mediaeval skeletal material. Journal of Archaeological Science 26: 675-685.

Stevens,R.E., Lister, A.M. and Hedges, R.E.M. (2006). Predicting diet, trophic level and palaeoecology from bone stable isotope analysis: a comparative study of five red deer populations. Oecologia 149 (1): 12-21.

Szpak,P., White,C.D., Longstaffe,F.J., Millaire, J.F. and Vásquez Sánchez, V.F. (2013). Carbon and Nitrogen Isotopic Survey of Northern Peruvian Plants Baselines for Paleodietary and Paleoecological Studies PLOS ONE 8:e53763

Tavares,P.C., Kelly,A., Maia,R., Lopes,R.J., Santos,R., Pereira,M.E., Duarte, A.C. and Furness, R.W. (2008). Variation in the mobilization of mercury into Black-winged Stilt *Himantopus himantopus* chicks in coastal saltpans, as revealed by stable isotopes. Estuarine, Coastal and Shelf Science 77: 65-76.

Traveling Luck. (2016). *'Traveling Luck for Allgold Creek, Yukon, Canada'.* Retrieved May, 3 2016 from http://travelingluck.com/North+America/Canada/Yukon/_5883985_Allgold+Creek.html

Vass, A.A. (2001). Beyond the grave: understanding human decomposition. Microbiology Today

Virtual Museum. (2016). *'Teachers; Centre'.* Retrieved February, 19 2016 from http://www.virtualmuseum.ca/edu/ViewLoitDa.do;jsessionid=6B3AFB6CD72689390383632B42270E0A method=preview&lang=EN&id=6799

Wilkinson,M.J., Yai, Y. and O'Brien, D.M. (2007). Age-related variation in red blood cell stable isotope ratios (delta13C and delta15N) from two Yupik villages in southwest Alaska: a pilot study. International Journal of Circumpolar Health, 66 (1).

Yamada,M., Tohno,S., Tohno,Y., Minami,T., Ichii, M. and Okazaki, Y. (1995). Accumulation of mercury in excavated bones of two natives in Japan. Science of the Total Environment 162 (2,3): 253-256.

Zwanziger, H. (1989). The multielemental analysis of bone. Biological Trace Element Research 19: 195-232.

3.8 Figures

Figure 3.1: Yukon Territory with Herschel Island. (http://www.virtualmuseum.ca/edu/)

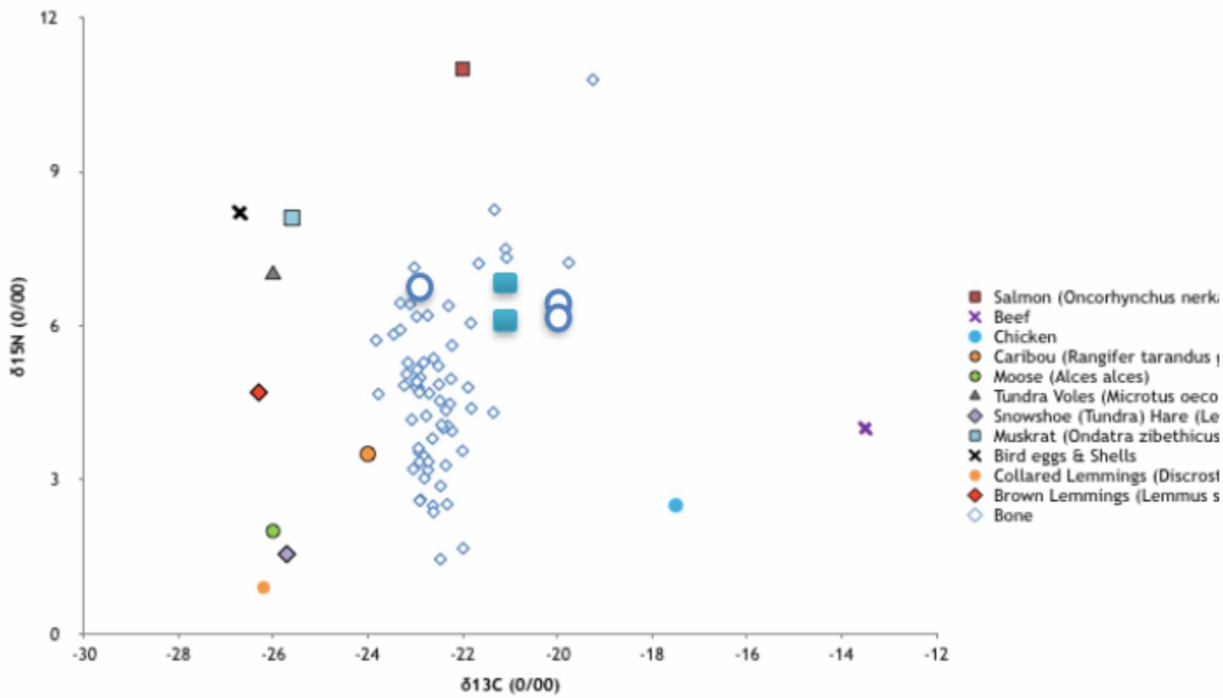

Figure 3.2: $\delta^{13}C$ stable isotope values of bone collagen as an indication of diet. Blue colored squares are Yukon Territory Arctic foxes. Open large circles are Yukon Territory red foxes. (Small blue diamonds represent Bethel red foxes only from unpublished data). Potential food sources data from Hobson et al. 2000, Neil and Cornwell 1992, Roth et al. 2007, Schmutz and Hobson 1998, Wilkinson et al. 2007).

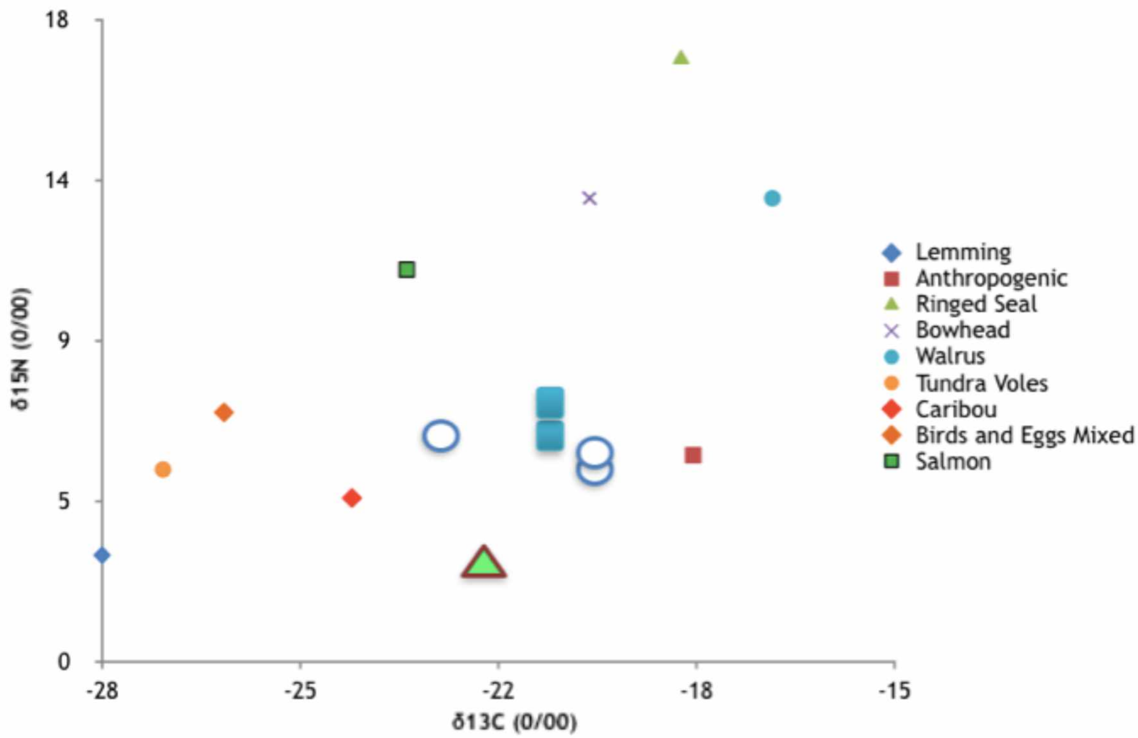

Figure 3.3: $\delta^{15}N$ stable isotope values of bone collagen an indication of trophic level. Blue colored squares are Yukon Territory Arctic foxes (n=2). Open large circles are Yukon Territory red foxes (n=3). Large green triangle with red border represents Bethel red foxes (n=65). Marine and terrestrial trophic level data from Hobson et al. 2000, Neil and Cornwell 1992, Roth et al. 2007, Schmutz and Hobson 1998, Wilkinson et al. 2007, and Anthropogenic (representing humans eating C4 plants) data from Leconte et al. 2011.

3.9 Tables

Table 3.1: Yukon Fossil Collection shows the THg concentrations in milligrams per kilogram (mg/kg) for individual samples and stable isotope values for $\delta^{13}C$ and $\delta^{15}N$ in parts permil (‰) for individual samples

Identification	Species	Specimen Type	Locality	THg Concentration*	$\delta^{13}C$	$\delta^{15}N$
YG 12.49	Alopex lagopus	right femur	Hershel Island	0.017	-21.13	5.59
YG 155.2	Alopex lagopus	right mandible	Hershel Island Beach	0.025	-21.36	7.22
YG 97.3	Vulpes vulpes	right tibia	Allgold Creek	0.036	-20.18	6.10
YG 97.4	Vulpes vulpes	left femur	Allgold Creek	0.073	-20.05	6.66
YG 130.65	Vulpes vulpes	right femur	Quartz Creek	0.010	-23.12	6.57

* Level of detection is 0.008 ug/g

General Conclusions and Future Direction

This dissertation examined how global climate changes can potentially impact the ecosystems of the red foxes of Bethel Alaska and the red and Arctic foxes of the Yukon Territory in Canada. This sentinel species' research has generated data from total mercury (THg) and stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of bone, hair, muscle, liver, kidney cortex and kidney medulla. In Chapter 1 the bone and hair data was used to determine if they could be predictors for THg concentration levels in the internal soft tissues; that is to say, their usefulness as indicators of contaminants in the wildlife of the Arctic ecosystem. Chapter 2 focused on $\delta^{13}C$ stable isotope data to determine the carbon source of the diet of the same red and Arctic foxes. The $\delta^{15}N$ stable isotope data was used for trophic level determination of these same foxes. In Chapter 3, I used archival fossil bone in a forensic study to create a historic baseline for THg concentration levels (health) and $\delta^{13}C$ and $\delta^{15}N$ values (dietary and trophic levels) in Arctic and red foxes from the Yukon Territory in Canada.

Chapter 1 is the first data set reported on red foxes for the state of Alaska. The results from Chapter 1 showed that there were no significant differences between male and female red foxes, from Bethel, Alaska, for THg concentrations in bone, hair, muscle, kidney cortex, kidney medulla, and liver. This suggests that the relationship between soft tissue THg concentrations and hair/bone THg concentrations is similar for these male and female foxes.

Hair was found to have a strong correlation with the other tissues. Whereas in this study, bone had a weak correlation with other tissues. The hair correlation may be strong enough to be used to predict THg concentrations in the kidney cortex ($R^2 = 0.61$) and liver ($R^2 = 0.63$). But, hair THg concentration was weakly predictive of kidney medulla ($R^2 = 0.50$) and muscle ($R^2 = 0.39$) THg concentrations. Bone THg concentration had no predictive value for liver ($R^2 = 0.24$), renal cortex ($R^2 = 0.35$), or renal medulla ($R^2 = 0.25$) THg concentrations. Bone THg concentration is weakly predictive of muscle ($R^2 = 0.40$) THg concentration (Dainowski et al., 2015).

Establishing this data set for these tissues allowed us the ability to compare our study with other studies. This study of the soft tissues revealed that these Alaskan free-ranging red foxes were eating at a higher trophic level than free-ranging red foxes from Italy (Corsolini et al., 1999) for both males and females. Their study differed from ours in regard to sex and age of the fox. We did not consider age in our study as we had very few foxes older than two. It is known that the body burden of THg usually increases with age. Red foxes from a fox fur farm in Poland (Cybulski et al., 2009) had liver and kidney (whole) THg concentrations higher than our Alaska free-ranging red foxes. This would be indicative of farm fed foxes, in which their feed possibly contained fish and or corn products. Free-ranging red foxes from Slovak Republic (Piskorová et al., 2003) showed much lower THg concentrations in liver, kidney (whole) and muscle, than Bethel Alaska red foxes. In Croatia, for both suburban and rural areas (Bilandzic et al., 2010), the red foxes were lower in their THg concentrations than these free-ranging red foxes in Alaska. In comparing bone THg concentrations, our Alaska free-ranging red foxes were found to be approximately the same as those red fox bones from Poland (Lanocha et al., 2012) and Spain (Millan et al., 2008).

We found that overall, the Western Alaska red fox mercury levels were higher than reported for other regions, mostly European. The results from this study confirmed the potential use of trapped animals, specifically foxes, as useful Arctic sentinel species to inform researchers about patterns in THg concentration levels over time as industrialization of the Arctic continues. A stable isotope study could support this finding of Alaskan red foxes eating at a higher trophic level. My findings suggest that THg levels in species in rural habitats may be more complicated than once believed.

Chapter 2 is the first report on the red fox stable isotope ratios and $\delta^{13}C$ and $\delta^{15}N$ values from wild foxes in Western Alaska. In this study I describe the C:N ratios in male and female red foxes from Bethel Alaska, evaluated the carbon and nitrogen correlations and related the wild red fox trophic level to potential mercury biomagnifications as reported in our previous THg study (Dainowski et al., 2015).

The C:N ratio demonstrated what was expected for foxes eating in terrestrial environments (Post et al., 2007) and the 1% difference in $\delta^{13}C$ means indicated there was little difference between the values for lipid extracted and non-lipid extracted tissues. No significant difference was found between males and females in the means and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ values and C:N ratios for all tissues.

The stable isotope signals from hair were highly correlated with the stable isotope profiles of some tissues in the same population. Bone showed a low to no correlation with some tissues. This finding suggests that hair from the red fox can be used as an indicator from a sentinel species for predicting some internal tissue profiles.

The red foxes from Bethel Alaska were compared to captive red and Arctic foxes (Roth and Hobson, 2000). We found that the Alaska red foxes differed in trophic position with the farmed red and Arctic foxes based on the $\delta^{15}N$ profile differences with their selection of prey or diet. Captive red foxes had a higher $\delta^{15}N$ values in muscle, liver and hair; whereas, wild red foxes had higher $\delta^{13}C$ values in muscle, liver and hair. This research has found, and also supports previous works (Peterson and Fry, 1987; Roth and Hobson, 2000), that the pellet diets of captive foxes, which contain carbohydrate and marine products, result in higher $\delta^{15}N$ values. The wild red foxes from Alaska have a different diet, they feed mostly on microtones (herbivores such as lemmings) that have a diet of C_3 plants (Dainowski et al., 2015; Savory, 2013) as well as a small amount of fish, frogs and birds when lemmings are scarce. This thesis supports that our red foxes mean nitrogen isotope ratio were 5.20‰ for muscle, which is between the value of brown lemmings (4.70‰) and tundra voles (7.00‰) (Dehn et al., 2006; Tarroux et al., 2010).

It has been shown that the $\delta^{15}N$ values for the hair and muscle (3.10‰) of the red foxes from Prudhoe Bay, Alaska (Savory et al., 2014) are feeding at a lower trophic level than the Bethel Alaska red foxes. This might be due to the proximity of a human industrial settlement, where the red foxes are feeding on anthropogenic foods.

The $\delta^{13}C$ and $\delta^{15}N$ stable isotope correlations between hair and other tissues were significant.These correlations between tissues gives us answers of the diet and trophic levels of wild foxes which can be very useful in forensic research.

The $\delta^{15}N$ stable isotope values helped to explain the THg levels that were found in Chapter 1 study (Dainowski et al., 2015). We also found a correlation between THg concentrations and $\delta^{15}N$ values in the hair of red foxes from Bethel Alaska. This finding shows that as the $\delta^{15}N$ values increase, so does the THg concentration levels. This implies that the THg may be coming through the mixed diet of lemmings, aquatic, and/or avian route of these red foxes.

Therefore, we conclude from these findings that as climate changes impact the biological and physical composition of ecosystems, the trophic positions will change for free-ranging red foxes. We also conclude that trophic position data is a key metric for ecological risk assessments of THg.

In Chapter 3 we used the data from Chapters1 and 2 to apply this approach to a forensic study. We examined archival museum bone samples (Arctic and red fox) in The Yukon Fossil Collection from the Yukon Territory in Canada. We reported total mercury (THg) accumulation and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) levels in order to evaluate Yukon Territory fox's past feeding ecology and trophic levels in order to understand their changing environment. This Yukon Territory red fox sample collection was also compared to the red fox samples from Bethel, Alaska in Chapter 1 (Dainowski et al., 2015). In this regard we were able to initiate a baseline of information over time.

When Bethel red fox data is compared to the Yukon Territory collection, we see that the Yukon Territory red fox THg concentrations are about ten times higher for mercury, on average, than the red foxes from Bethel, Alaska. We found two red foxes from the Yukon Territory that had higher THg concentrations than the other Yukon Territory red or Arctic foxes, or Bethel red foxes. This may be due to the fact that these two particular Yukon Territory red fox bones were both from the Allgold Creek area. These areas are known for mining activities, which tend to leave mercury behind in the key stages of the mining process. This mercury in turn seeps into the local environment. Another possible explanation is that Allgold foxes were eating garbage which might have contained fish, an aquatic source high in mercury. The third Yukon Territory red fox was found to be eating a mammal diet with a C_3 plant pathway.

The THg concentration levels of the Yukon Territory Arctic foxes fell between both the Bethel and Yukon red foxes THg concentrations. This indicates these foxes were most likely eating a mixed diet composed of both lemmings and/or voles and an aquatic source high in mercury. Besides coming directly from rivers or creeks, an aquatic source of food, such as fish, THg can also come from anthropogenic food waste sources, such as garbage areas.

The $\delta^{13}C$ stable isotope values for both Arctic foxes, and two red foxes, from the Yukon Territory in Canada suggested an aquatic source high in mercury, possibly a salmon migration diet. The third red from the Yukon Territory tends toward a terrestrial mammal diet. The $\delta^{15}N$ stable isotope values supported this and confirm that the trophic level of the Yukon red and Arctic foxes are in line with a trophic level of an omnivore; however, they do tend toward a slight salmon diet. In comparison, the $\delta^{15}N$ values for red foxes from Bethel, Alaska indicates that these foxes are eating at a lower trophic level which could indicate a herbivore protein influence (Dainowski et al., 2015).

The findings from this forensic study had many unknown variables, so there are no conclusive answers. This is due to the fact that no information existed for these bones; such as, specifically, where were they found, age of bones,condition and storage of the bones, etc. Also, the sample size was very small, three (3) red fox bones, and two (2) Arctic fox bones. This by no means is a representative example of the entire Yukon Territory in Canada. Therefore it is impossible to have definitive answers, and proper statistical analysis that would be able to indicate key relationships connecting past populations to current day populations. Also, future soil samples would be of an importance to determine if any toxicants are in the local ecosystem or the result of long range transport. Finally, these forensic bones need to be tested for age in order to establish a correct baseline for THg contaminants; as well as, to determine if the foxes diet and/or trophic levels are changing over time.

However, this study is a good starting point for future forensic analysis of archival bone. We were able to show that museum specimens are valuable because 1) metals can be detected, 2) stable isotopes can be detected and 3) comparisons to extant populations can be performed. It is important to continue monitoring these species in order to evaluate how climate change is impacting the Arctic environment.

References

Bilandzic, N., Dezdek, D., Sedak, M., Dokic, M., Solomun, B., Varenina, I., Knezevic, Z., Slavica, A., 2010. Concentrations of trace elements in tissues of red fox (*Vulpes vulpes*) and stone marten (*Martes foina*) from suburban and rural areas in Croatia. Bull. Environ. Contam. Toxicol. 85, 486-491.

Corsolini, S., Focardi, S., Leonzio, C., Lovari, S., Monaci, F., Romeo, G., 1999. Heavy metals and chlorinated hydrocarbon concentrations in the red fox in relations to some biological parameters. Environ. Monit. Assess. 54, 87-100.

Cybulski, W., Chalabis-Mazurek, A., Jakubczak, A., Jarosz, L., Kostro, K., Kursa, K., 2009. Content of lead, cadmium, and mercury in the liver and kidneys of silver foxes (*Vulpes vulpes*) in relation to age and reproduction disorders. Bull. Vet. Inst. Pulawy 53, 65-69.

Dainowski, B.H., Duffy, L.K., McIntyre, J., Jones, P., 2015. Hair and bone as predictors of tissular mercury concentration in the Western Alaska Red Fox, *Vulpes vulpes.* Sci. Total Environ. 518-519, 526-533, doi:10.1016/j.scitotenv.2015.03.013.

Dehn, L.A., Follmann, E.H., Thomas, D.L., Sheffield, G.G., Rosa, C., Duffy, L.K., O'Hara, T.M., 2006. Trophic relationships in an Arctic food web and implications for trace metal transfer. Sci. Total Environ. 362, 103-123.

Lanocha, N., Kalisinska, E., Kosik-Bogacka, D.I., Budis, H., Noga-Deren, K., 2012. Trace metals and micronutrients in bone tissues of the red fox *Vulpes vulpes* (L., 1758). Acta Theriol. 57, 233-244.

Millan, J., Mateo, R., Taggart, M.A., Lopez-Bao, J.V., Viota, M., Monsalve, L., Camearero, P.R., Blazquez, E., Jimenez, B., 2008. Levels of heavy metals and metalloids in critically endangered Iberian lynx and other wild carnivores from Southern Spain. Sci. Total Environ. 399, 193-201.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18, 293-320.

Piskorová, L., Vasilková, Z., Krupicer, I., 2003. Heavy metal residues in tissues of wild boar (*Sus scrofa*) and red fox (*Vulpes vulpes*) in the Central Zemplin region of the Slovak Republic. Czech J. Anim. Sci. 48, 134-138.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179-189.

Roth, J.D., Hobson, K.A., 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. Canadian Journal of Zoology 78 (5), 848-852.

Savory, G., 2013. Foxes and Food Subsidies: Anthropogenic food use by red and arctic foxes, and effects on arctic fox survival, on the arctic coastal plain of Alaska. (MS Thesis), University of Alaska Fairbanks, Fairbanks, AK.

Savory, G.A., Hunter, C.M., Wooller, M.J., O'Brien, D.M., 2014. Anthropogenic food use and diet overlap between red foxes (*Vulpes vulpes*) and arctic foxes (*Vulpes lagopus*) in Prudhoe Bay, Alaska. Can. J. Zool. 92, 657-663.

Tarroux, A., Ehrich, D., Lecomte, N., Jardine, T.D., Bêty, J., Berteaux, D., 2010. Sensitivity of stable isotope mixing models to variation in isotope ratios: evaluating consequences of lipid extraction. Methods Ecol. Evol. 1, 231-241.